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The Quantitative Genetics of Prescutellar Bristles in Melon Fly, *Bactrocera cucurbitae* (Coquillett)

Edward Yun Cheng[1], Chih-Hsien Wang[2], Yu-Bing Huang[3,*], and Ming-Yao Chiang[3]

Abstract

Cheng, E. Y., C. H. Wang, Y. B. Huang, and M. Y. Chiang. 2014. The quantitative genetics of prescutellar bristles in melon fly, *Bactrocera cucurbitae* (Coquillett). J. Taiwan Agric. Res. 63(3):179–187.

The polymorphism of prescutellar bristles (*prsc*) in melon fly, *Bactrocera cucurbitae* (Coquillett), was investigated by artificial selection of phenotypes and quantitative genetic analysis. In the wild strain, generally more than 95% population have 2 *prsc* while some may have 4 *prsc*. By selection, however, a group of phenotypes was appeared with the number of *prsc* reach up to 14 and more. All multiplied bristles were randomly arranged in a region of posterior half of *scutum* and between the lateral *vitta*. The quantitative genetic analysis was conducted by crossing two strains of different phenotypes, one from each strain. In each cross only one virgin male and one virgin female were paired, with a total of 6 pairs in each experiment, and 200–500 offsprings from each experiment were examined for their phenotypes. For each cross, only one 2-*prsc* individuals (the major component of the wild population) and one 12-*prsc* or 14-*prsc* individuals from the selected strains were interbred, and then inbred F_1 for the F_2 progeny. The results showed that for 2-*prsc* × 12-*prsc*, the F_1 progeny constituted a group of phenotypes with different *prsc* numbers, and the mean was 4.7 ± 0.9 which was in-between two parental phenotypes. The mean of *prsc* in F_2 is 4.7 ± 1.9. The difference between the means of F_1 and F_2 is insignificant in the *t*-test ($P > 0.05$). For 2-*prsc* × 14-*prsc*, the F_1 progeny constituted a group of phenotypes, with the mean of 5.0 ± 1.0 which was in-between their parental phenotypes. The F_2 offsprings had their mean at 5.1 ± 2.3. The *t*-test indicated no significant difference between the means of F_1 and F_2 ($P > 0.05$). Moreover, as the coefficients of variation of F_2 were higher than those of F_1 (40.1% > 19.5% versus 45.9% > 21.7%), it suggests that more variability around the mean of F_2 than that of F_1. The extreme values in *prsc* for F_2 were extended to the range of their parental values (2–15 *prsc* and 1–17 *prsc*, respectively) than did the extreme values of F_1. Results of this study were similar to that reported by Emerson & East (1913), indicating that the *prsc* of melon fly is a continuous and quantitative trait in inheritance.

Key words: *Bactrocera cucurbitae*, Prescutellar bristles, Quantitative genetic analysis, Quantitative trait.

INTRODUCTION

The melon fly, *Bactrocera cucurbitae* (Coquillett), is an economic important pest in agriculture, and identifying this tephritidae species is a routine practice in the integrated control program. The thorax bristles are important morphological characters in the fruit fly identification. For example, the appearance of scutellar bristles in thorax has been suggested by Drew & Hancock (2001) in an attempt to determine which characters are primitive within the Da-

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cini. White (2001) described that the prescutellar bristles (*prsc*) can be used as one of the characters in classifying subgenera of *Bactrocera* and *Dacus*. Particularly, for the adult identification of melon fly, White & Elson-Harris (1992) described that a pair of *prsc* and 2 or 4 (rarely) scutellar bristles were present.

In 1996, a field survey of melon fly in Taiwan revealed that the majority possesses a pair of *prsc* as that reported by White & Elson-Harris (1992). However, there were 5% of the population otherwise, and 0 to 4 *prsc* other than 2 *prsc* were found in them. This *prsc* polymorphism had caused confusion in the specimen identification and demanded further clarification. The insect bristles are external sensory organs of the peripheral nervous system. Any change in their number will involve the basic innervation difference in the gene controlled embryonic development. For example, in *Drosophila*, the number of bristles has been a classic quantitative genetic character (Mackey 1995), and the development of bristles is associated with the expression of genes of the achaete-scute complex (ASC) in the epidermis of *Drosophila* (Brakefield *et al*. 2003). By artificially selected the numbers of sternopleural and abdominal bristle in the laboratory established wild Drosophila strain, the marker genotypes were analyzed by quantitative trait loci (QTL) mapping techniques for their phenotype (Long *et al*. 1995; Lynch & Walsh 1998; Gurganus *et al*. 1999). Do the melon flies have the *prsc* polymorphism? How is it controlled genetically? Both are the purposes of this study.

MATERIALS AND METHODS

The caged melon flies were reared at room temperature, on a diet of agar water gel, protein hydrolyze and sugar. The fresh cucumber was used for egg laying. The morphology of melon fly was observed under a dissecting microscope. The intra-alar bristles were not included in the *prsc* count (Appendix). The live specimens were either immobilized by low temperature or restrained inside a glass vial for *prsc* phenotype inspection. The morphological description and nomenclature of Tephritidae by Drew & Hancock (1994) was adopted in this study.

The laboratory reared wild (LRW) strain

A LRW-strain was established by harvesting the larvae from field infested cucumbers and reared in the laboratory.

The artificial selected strains

Strains with different *prsc* numbers were selected artificially. In the selection, the melon flies were harvested at pupal stage, and each pupa was transferred into a glass vial. Every day, the freshly emerged flies were inspected for sexes and phenotype of *prsc* before being selected for pairing. The successive selection was conducted by pairing the desired phenotype from generation to generation, and each selection consisted of six pairs.

Characterizing the strains in regard of *prsc*

For each strain around 500 to 1000 flies of each selected generation were inspected for their *prsc* numbers. The *prsc*-phenotypes appeared in a continuously distributed trait, the mean, the standard deviation and the range of *prsc* were used to define the character of a strain.

Genetic studies

To be precise in the genetic study, only one desired phenotype from each strain was selected for crossing. The genetic study was carried out by crossing two different phenotypes from two different strains, and then inbred F_1 for the F_2 progeny. All experiments consisted of six pairs, and 200 to 500 offsprings from each replicate were examined for the *prsc* number count.

Statistical analysis

The *F*-test and the *t*-test were used to vali-

date the significance of difference between any two strains. All *prsc* results were separately recorded according to male and female.

RESULTS

The strains and the selection results

The LRW strain has five phenotypes. The majority (96.1%) of the population has 2 *prsc*, while 0.2% had no *prsc*, 1.0% had one *prsc*, 2.4% had 3 *prsc* and 0.3% had 4 *prsc* (Fig. 1A). The mean value of the LRW strain was 2.0 ± 0.2 in the range of 0–4 *prsc*. The phenotype constitute of this strain was frequently examined to assure that the *prsc* character of wild strain was maintained.

The artificial selection was to establish the mono-phenotype strains of both the 2-*prsc* and 4-*prsc* phenotypes.

The 2-*prsc* strain

After one hundred generations, the mono-phenotype strain could not be selected. The 2-*prsc* phenotype can only be improved from 96.1% to 97.3%, and the difference from the LRW-strain is not significant ($P > 0.05$), though both the 0- and the 4-*prsc* phenotypes were eliminated, and only the 1- to 3-*prsc* phenotypes remained at 1% and 1.6%, respectively (Fig. 1B). The mean value of the 2-*prsc* strain was 2.0 ± 0.2 in the range of 1–3 *prsc*.

The 4-*prsc* strain

The melon fly responded to the 4-*prsc* selection rapidly (Fig. 2). The 2-*prsc* phenotype decreased from 95% to less than 5% within 3 generations, and further lowered to less than 0.1% in 10 generations. The 4-*prsc* phenotype increased from 0.1% to 70% in 3 generations, and then reached a steady state between 75% and 85% of the population. The population hosts a continuous distributed trait of *prsc* phenotypes. At the end of 90 generations of selection, the 1-, the 2-, the 3-, the 4-, the 5-, the 6-, and the 7-*prsc* phenotypes were 0.1, 0.6, 5.5, 84.7, 9.3, 0.4, and 0.1% of the population,

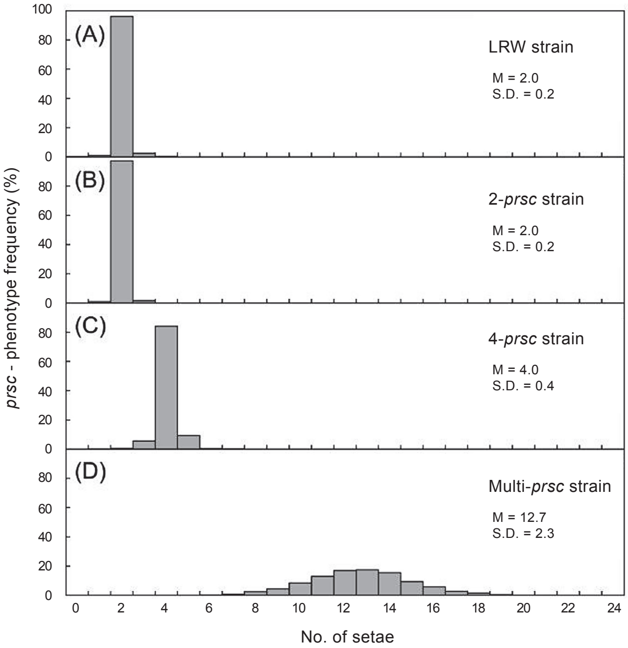


Fig. 1. The prsc-phenotype distribution of (A) the laboratory reared wild strain (LRW); (B) the selected 2-*prsc* strain; (C) the selected 4-*prsc* strain; and (D) the selected multiple-*prsc* strain. *prsc*: prescutellar bristles.

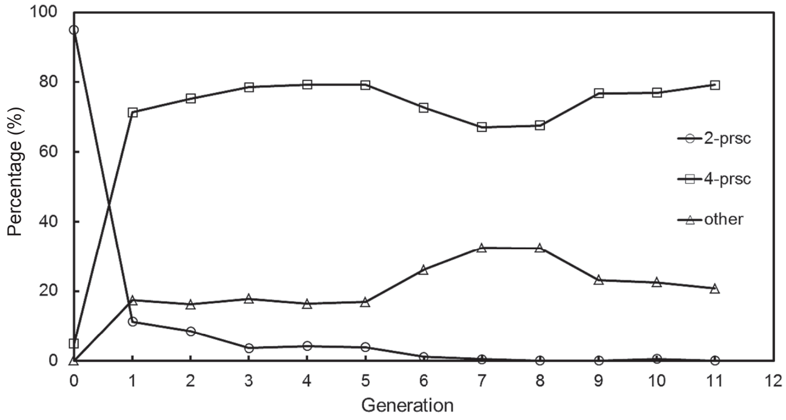


Fig. 2. The frequency variation of different *prsc*-phenotypes in the first ten generations selection of the 4-*prsc* strain. *prsc*: prescutellar bristles.

respectively (Fig. 1C). The mean value of the 4-*prsc* strain was 4.0 ± 0.4 in the range of 1–7 *prsc*.

Both the 2-*prsc* and the 4-*prsc* strain selections proclaimed that it was not possible to select a strain with only one phenotype or the mono-phenotype. Since more phenotypes appeared in the selection, it led to a decision of selecting a strain with more *prsc* or the multiple-*prsc* strain.

The multiple-*prsc* strain

The selection of multiple-*prsc* strain was preceded by successively pairing the individuals with the highest number of *prsc* from generation to generation. And after 40 generations, a colony of melon flies with multiple *prsc* in continuous traits was selected (Fig. 1D). The highest frequency is 17.5% for the 13-*prsc* phenotype, while the 12- and the 14-*prsc* phenotypes were 17.0 and 15.6%, respectively. For the multiple-*prsc* strain, the mean was 12.3 ± 1.9 in the range of 8–19 *prsc*.

The stability of the multiple-*prsc* strain was examined by lifting the selection for 10 generations, and the mean remained almost unchanged, i.e., mean = 12.7 ± 2.3 with the range of 5–23 *prsc*. The increase of variance is due to the range expansion of phenotypes. The stable result suggests that the strain is homozygous for the genes controlling *prsc*. The advantage to have the multiple-*prsc* strain is that it provides multiple choices of phenotypes for the genetic study.

In morphology, the *prsc* only multiplied in a region of the posterior half of *scutum* and between the *lateral postsutural vitte* (Plates A, B, C & D). The distribution of multiplied *prsc* in posterior *scutum* is symmetric but without a fixed pattern as the analysis of 600 flies of the multiple *prsc* strain in three repeats (Table 1). Simpson *et al*. (1999) suggested that the spatial distribution of sensory bristles on the notum of different species of *Diptera* is that the species displaying ancestral features have a simple organization of random distribution, which may also be applied to the primitive stage of bristle development in selected strains.

The result of artificial selection has demonstrated that the polymorphism of *prsc* is evident in the melon fly and cannot be eliminated by selection. All *prsc* test results showed that there is no difference between male and female, hence genetically there is no sex linkage involved in the *prsc* determination.

Genetic study

In *prsc* selections, the continuous trait in the form of a group of phenotypes always appeared, and the repeated phenomena implicate

Table 1. The left vs. right symmetric analysis of *prsc* (prescutellar bristles) distribution in the scutum of multiple-*prsc* strain melon flies.

Test no. (N = 200)	No. of *prsc* ± S.D.			
	Left	Right	*t*-value	*P*-value
1	6.3 ± 1.4	6.2 ± 1.4	0.085	0.93*
2	6.3 ± 1.5	6.4 ± 1.3	0.854	0.39
3	6.3 ± 1.5	6.3 ± 1.5	-0.331	0.74

* The differences of all three replicates are not significant at $P > 0.05$ level.

that the quantitative genetics is the possible control mechanism for *prsc* in melon fly. In order to search for more credible evidences, a classic example of quantitative genetic analysis of corn ear length by Emerson & East (1913) was simulated. By crossing two distinctly different phenotypes from two strains, both were stable and presumed to be homozygous for the genes controlling *prsc*. And then the interbred F_1 to the F_2 generation should provide the analytic evidences for quantitative genetics.

The first test is the cross between the 2-*prsc* and the 12-*prsc* phenotypes

The 2-*prsc* individuals from the 2-*prsc* strain and the 12-*prsc* individuals from the multiple-*prsc* strain were chosen for the cross. Fig. 3 presents the phenotypes of parents, F_1 progeny and the inbred F_2 progeny, respectively, in frequency histograms. The F_1 progeny constituted a group of phenotypes with *prsc* number ranged 2–8 and the mean of 4.7 ± 0.9 which was in-between two parental phenotypes. The mean of *prsc* in F_2 was 4.7 ± 1.9 and the range was 2–15 *prsc*. The difference between the means of F_1 and F_2 is insignificant in the *t*-test. The greater variance of F_2 than F_1 is due to the extreme value of F_2 phenotypes extended to two parental strains.

The 2nd test, the cross of the 2-*prsc* and the 14-*prsc* phenotypes

The 2-*prsc* individuals from the 2-*prsc* strain and the 14-*prsc* individuals from the

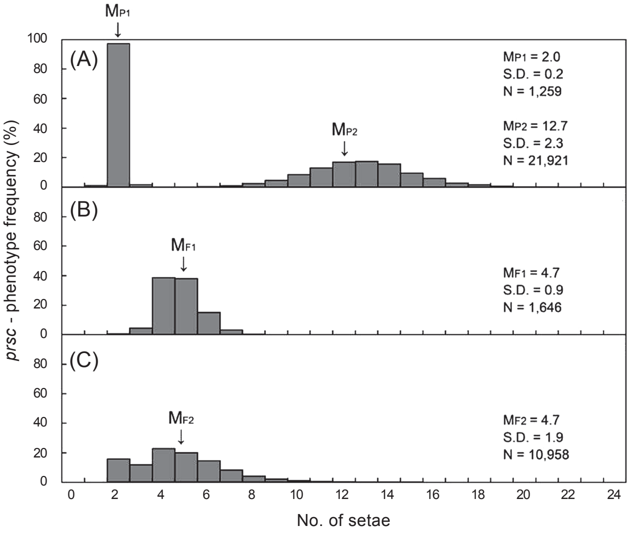


Fig. 3. Histogram of phenotypes frequencies with different numbers of *prsc* from (A) the parental strains, (B) the F_1 and (C) the F_2 generations in the 2-*prsc* × 12-*prsc* phenotypes cross test. *prsc*: prescutellar bristles.

multiple *prsc* strain were chosen for the cross. The Figs. 4A, B and C present the phenotypes of parents, F_1 progeny and the inbred F_2 progeny in frequency histograms. The F_1 progeny constituted a group of phenotypes ranged 1–9 *prsc* with the mean of 5.0 ± 1.0 which was in-between two parental phenotypes. The F_2 offsprings had the mean at 5.1 ± 2.3 and the range was 1–17 *prsc*. As the 1st test, the *t*-test concluded that the difference between the means of F_1 and F_2 is not significant. The greater variance of F_2 is due to the extreme value of phenotypes extended to two parental strains.

In both tests, results were similar to quantitative inheritance study reported by Russell (2005): (1) The mean value of the quantitative trait, or the *prsc* in the F_1 progeny was intermediate between the means of the two parental phenotypes. (2) The mean value of the *prsc* in the F_2 progeny was approximately equal to the mean for the F_1 population. (3) When standard derivation was compared, the F_2 showed more *prsc* variability around the mean than the F_1 did. (4) The extreme values for the *prsc* in the F_2 extended to the two parental values than did the extreme values of the F_1.

DISCUSSION

The polymorphism of thorax *prsc*-bristles is found in melon fly, and the 2-*prsc* phenotype is dominated at 95% in the wild population of Taiwan. The initial attempt is to select the mono-phenotype lines for the genetic study. However, it was the continuous trait of phenotypes appeared in the selection, a mono-phenotype strain was an in vain attempt. The effort was redirected to select melon fly with more *prsc* for the genetic investigation. Three strains were selected from the LRW strain, which itself is a strain with a narrow ray of phenotypes. The selection only moves a window, or the mean, along a continuous trait to a certain selected point. The strictness of selection enforced direct influences on the variation and the range of phenotypes in progeny. For example, when the 12-*rs* individuals were replaced by the 14-*prsc* individuals in the second genetic test, the mean of *prsc* in F_1 increased from 4.7 to 5.0. The result of directional selection revealed that the *prsc* numbers in melon fly were greater than those of wild populations (Fig. 5). Various phenotypes and their distribution patterns offered a chance for genetic

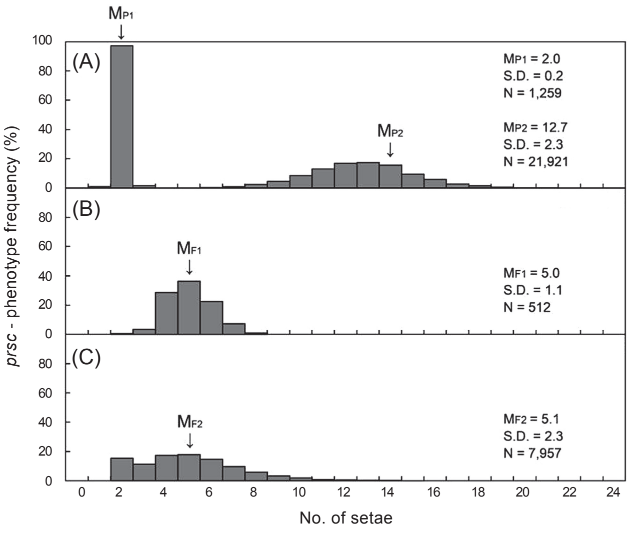


Fig. 4. Histogram of phenotypes frequencies with different numbers of *prsc* from (A) the parental strains, (B) the F_1 and (C) the F_2 generations in the 2-*prsc* × 14-*prsc* phenotypes cross test. *prsc*: prescutellar bristles.

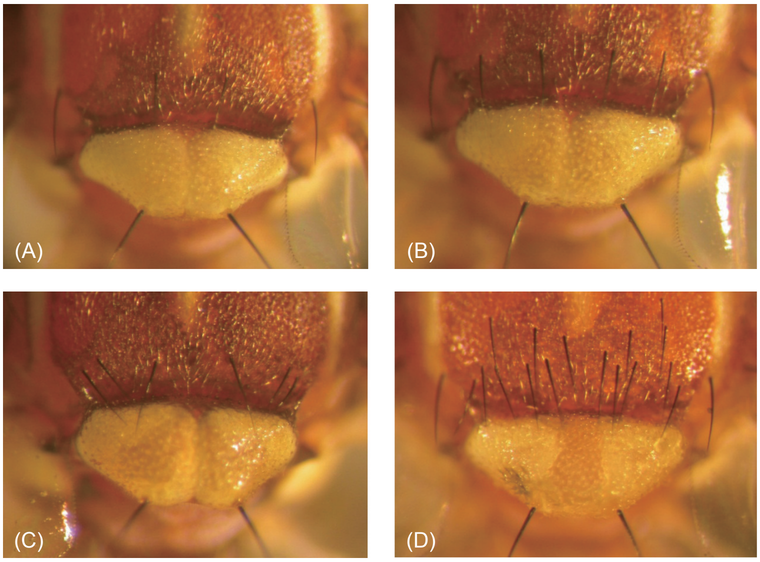


Fig. 5. Melon flies with various numbers of *prsc* by selection. (A) The 2 *prsc* phenotype or the wild type; (B) The 4 *prsc* phenotype; (C) The 6 *prsc* phenotype; and (D) The 16 *prsc* phenotype.

investigation on possible control mechanism.

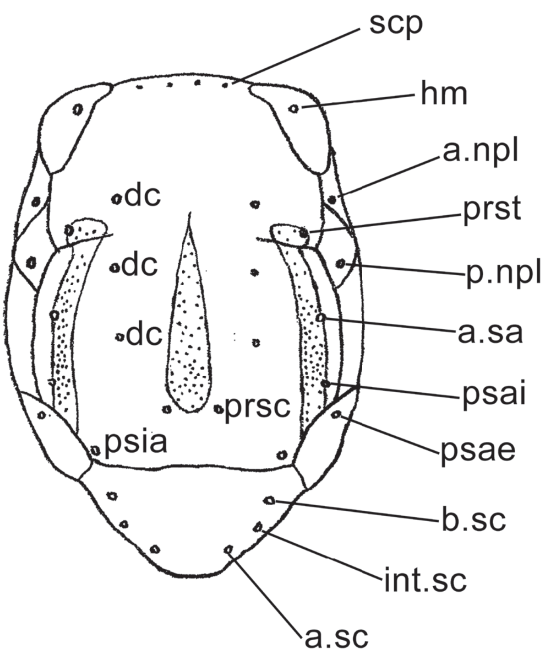
The quantitative genetics of *prsc* became in sight because a tendency of normal distribution of *prsc* phenotypes is evident: the more or the less *prsc* number phenotypes from the center or major phenotype were always gradually lowered in frequency count and formed a bell shape distribution. Hence, the mean, the standard deviation, and range of *prsc* were suitable to characterize the strain as well as for the genetic analysis.

The genetic analysis of mean, variance and the range of *prsc* in F_1 and F_2 of both 2-*prsc* × 12-*prsc* and 2-*prsc* × 14-*prsc* crosses fit the criteria of corn ear length study of Emerson & East (1913); hence, confirming that the quantitative genetic mechanism is responsible for the *prsc* numbers in melon fly. Why the 2-*prsc* phenotype dominates the wild population at a percentage more than 95% and whether this fact implies that the contribution of the polygenes in the continuous trait may not be equal in their dominance are interesting. This possibility is evident in the genetic studies, as the *prsc*-phenotype distributions of F_2 were slightly deviated from the normal distribution, in favor of 2-*prsc* phenotype or the dominated wild type. The environmental influence, an important consideration in quantitative genetics, is minimum due to all melon flies were reared in the same laboratory condition with uniform diets, temperature, humidity, photoperiod, etc..

CONCLUSIONS

This investigation is tried to find out the genetic control mechanism of *prsc* number in the thorax of melon fly. Through the directional selection of strains with different phenotypes and the genetic tests by crossing different phenotypes for F_1 through F_2, it is possible to conclude that *prsc* is controlled by quantitative genetics based on following results: (1) In F_1 progeny, the mean of *prsc* is intermediate between two parental phenotypes. (2) The mean of *prsc* in the F_2 progeny is statistically equal to the mean of F_1. (3) The standard deviation of F_2 showed more variability around the mean of *prsc* than the F_1 did. (4) The extreme values for the *prsc* in the F_2 progeny extended to the two parental values than did the extreme values of the F_1.

APPENDIX



Appendix The schematic setae of mesonotum for the melon fly.

scp: scapular bristles; *hm*: humeral bristle; *a.npl*: anterior notopleural;
prst: praestutural bristles; *p.npl*: posterior notopleural bristles;
a.sa: anterior supra-alar bristles; *psai*: posterior supra-alar bristles;
psae: posterior external supra-alar bristles; *prsc*: prescutellar bristles;
psia: posterior intra supra-alar bristles; and *b.sc*: base scutellar bristles.

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瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 中胸背板剛毛之數量遺傳機制

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摘要

鄭允、王志賢、黃毓斌、江明耀。2014。瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 中胸背板剛毛之數量遺傳機制。台灣農業研究 63(3):179–187。

野生瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 中胸背板之剛毛數 (prescutellar bristles; *prsc*) 有 95% 個體為 2，此外尚有少數個體為 0–4 隻剛毛，合計約 5%。為瞭解此多型態 (polymorphism) 之控制機制，以室內汰選方式進行 2-*prsc* 及 4-*prsc* 單一形態型品系之純化，經過一百代以上，仍無法選出，反而在 4-*prsc* 汰選中觀察到有更多剛毛個體的出現。而 2-*prsc* 之汰選結果平均數為 2.0 ± 0.7，範圍為 1–3；4-*prsc* 之結果平均數為 4.0 ± 0.4，範圍為 1–7，因此再汰選多剛毛品系，所得結果為平均數為 12.7 ± 2.3，範圍為 8–19。所有汰選均得到由一系列連續分布的多形態個體所組成之族群，此等多形態型組成有可能為數量遺傳 (quantitative genetics) 機制所造成。因此，進行遺傳測試求證，以 2-*prsc* 為父本，分別與 12-*prsc* 及 14-*prsc* 之母本雜交出 F_1 子代後再自交成 F_2 子代，計算其 *prsc* 不同數目型之個體數，求取平均值、標準誤差及分布範圍。F_1 與 F_2 平均值以 *t*-test 進行差異顯著度測試，結果發現下列四項數量遺傳之佐證：(1) 兩項測試之 F_1 平均剛毛數分別為 4.7 及 5.0，均介於原父本及母本之間；(2) 兩項測試之 F_2 平均剛毛數分別為 4.7 及 5.1，在 *t*-test 顯示與 F_1 之平均值相同無顯著差異；(3) F_2 子代剛毛數變異係數 (Coefficient of variation) 均較 F_1 子代者為大，分別為 40.1% > 19.5% 及 45.9% > 21.7%；(4) F_2 子代剛毛數分佈之兩端極端可達父本及母本之範圍，分別為 2–15 *prsc* 及 1–17 *prsc*。本研究與 1913 年 Emerson 及 East 之玉米穗長度數量遺傳之經典測試結果均能吻合。因此，推論瓜實蠅中胸背板剛毛數之控制機制為數量遺傳，也與另一雙翅目之 *Drosophila* 屬剛毛數控制機制為數量遺傳相同。

關鍵詞：瓜實蠅、中胸背板剛毛、數量遺傳分析、數量策略。

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Revision of the Genus *Sangariola* Jacobson (Coleoptera: Chrysomelidae: Galerucinae: Alticini)

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Abstract

Lee, C. F. 2014. Revision of the genus *Sangariola* Jacobson (Coleoptera: Chrysomelidae: Galerucinae: Alticini). J. Taiwan Agric. Res. 63(3):188–204.

Five species of *Sangariola* Jacobson are recognized as valid. *Sangariola hirashimai* Kimoto, 1970, *Sangariola fortunei* (Baly 1888), and *Sangariola punctatostriata* (Motschulsky 1861) are redescribed. *Sangariola costata* Chûjô, 1935b is removed from synonymy with *S. punctatostriata* (Motschulsky 1861). A new species, *Sangariola yuae* sp. nov., is described from Taiwan. *Charidea nigrosuturalis* var. *obliterata* Pic, 1937 is proposed as a new synonym of *S. fortunei* (Baly 1888). Lectotypes are designated for *Charidea fortunei* Baly, 1888, *Charidea nigrosuturalis* Pic, 1937, *Galleruca* (sic!) *punctatostriata* Motschulsky, 1861, *Galeruca* (*Adimonia*) *multicostata* Jacoby, 1885, and *Sangariola costata* Chûjô, 1935b.

Key words: Taxonomy, Flea beetle, *Lilium*.

INTRODUCTION

The genus *Sangariola* was erected for *Galeruca punctatostriata* Motschulsky, 1861 by Jacobson (1922). Although the genus *Charidea* was erected earlier (Baly 1888), it is an unavailable name because of the junior homonymy of *Charidea* Dalman, 1816 (Lepidoptera: Thyrididae). Another genus, *Allophyla* Weise, was erected earlier (Weise 1889), but it also is an unavailable name because of the junior homonymy of *Allophyla* Loew, 1864 (Diptera: Heleomyzidae). Hincks (1949) apparently did not know about publication of the available name (*Sangariola*). He proposed an unnecessary replacement name, *Lophallya*, for *Allophyla*.

Sangariola currently comprises three valid species: *Sangariola punctatostriata* (Motschulsky 1861), *Sangariola fortunei* (Baly 1888), and *Sangariola hirashimai* Kimoto, 1970. However, a number of names have been synonymized with these species. *Galeruca* (*Adimonia*) *multicostata* Jacoby, 1885, *Charidea regularis* Pic, 1928, *Sangariola costata* Chûjô, 1935b, and *S. punctatostriata aequicostata* Chûjô, 1938 were placed as synonymies of *S. punctatostriata* (Motschulsky 1861). *Allophyla auora* Weise, 1889, *Charidea bicostata* Pic, 1928, *Charidea nigrosuturalis* Pic, 1937, *Charidea pieli* Pic, 1937, *Charidea bicostata* var. *atriceps* Pic, 1938, and *Sangariola fortunei* var. *unicoloripennis* Chûjô, 1941 were synonymized with *S. fortunei* (Baly 1888). Taxonomic statuses of all available names were reevaluated through type examinations.

S. punctatostriata and *S. fortunei* were re-

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ported to feed on leaves of *Lilium* spp. (Chûjô & Kimoto 1961; Yu 1996) used as cut flowers commercially. All *Sangariola* species occur in Taiwan (Kimono & Chu 1996). Specimens of the genus have been extensively collected and studied by the Taiwan Chrysomelid Research Team (TCRT), and food plants documented. The results are presented here. The TCRT was founded in 2005 and comprises ten members. All are amateurs interested in inventorying all species of Chrysomelidae in Taiwan.

MATERIALS AND METHODS

Nearly two hundred specimens and types have been examined. Most of them were collected by members of the TCRT and deposited at the Taiwan Agricultural Research Institute (TARI). Others are deposited in the historical collection at the same institute. To prepare drawings of the adult reproductive systems, the abdomens of adults were separated, boiled in a 10% KOH solution, cleared in distilled water, and then mounted on microscope slides in glycerin for observation. Drawings were made using a Leica M165 stereomicroscope or a Nikon ECLIPSE 50i compound microscope. Body parts were then stored in glycerin tubes with the dry mounted specimens.

Host plants were recorded by observing adult feeding behavior in the field. Plants were identified by Chih-Kai Yang.

The type specimens examined are deposited in the following collections: BMNH [Natural History Museum, London, United Kingdom (Michael Geiser)]; BPBM [Bernice P. Bishop Museum, Hawaii, USA (Shepherd Myers)]; CAS [California Academy of Sciences, California, USA (David H. Kavanaugh)]; KMNH [Kitakyushu Museum of Natural History, Kitakyushu, Japan (Kyoichiro Ueda)]; KUEC [Kyushu University, Fukuoka, Japan (Munetoshi Maruyama)]; MCZC [Museum of Comparative Zoology, Harvard University, Massachusetts, USA (Philip D. Perkins)]; MNHN [Muséum National d'Histoire Naturelle, Paris, France (Antoine Mantilleri)]; NHRS [Naturhistoriska Riksmuseet, Stockholm, Sweden (Johannes Bergsten)]; NMNS [National Museum of Natural Science, Taichung, Taiwan (Ming-Luen Jeng)]; TARI (Taiwan Agricultural Research Institute, Taichung, Taiwan); ZMUM [Zoological Museum, Moscow State University, Russian (L. N. Medvedev)].

Exact label data are cited for all type specimens; a double slash (//) divides the data on different labels and a single slash (/) divides the data in different rows. Other comments and remarks are indicated as follow: (p)- preceding data are printed, (h)- preceding data are handwritten, (w)- white label, (y)- yellow label, (b)- blue label, and (r)- red label.

RESULTS AND DISCUSSION

Genus *Sangariola* Jacobson, 1922

Charidea Baly 1888: 157. Type species: *Galeruca punctatostriata* Motschulsky, 1861. (junior homonym of *Charidea* Dalman, 1816).

Allophyla Weise 1889: 624. Type species: *Allophyla* aurora Weise, 1889. (junior homonym of *Allophyla* Loew, 1864).

Sangariola Jacobson 1922: 522. Type species: *Galeruca punctatostriata* Motschulsky, 1861.

Lophallya Hincks 1949: 616. (replacement name for *Allophyla* Weise).

Diagnosis (modified after Konstantinov & Vandenberg 1996). Body large, elongate, dorsally flattened. Color reddish or dark reddish brown.

Head oval, more or less convex in lateral view. Frontal ridge replaced by short longitudinal furrow between antennal sockets. Antennal calli longitudinal, raised, triangular, broadly

connected. Orbital line deeply impressed. Eye small. Interantennal space subequal to diameter of antennal socket. Antenna 11-segmented, filiform.

Pronotum more or less narrow, flat with transverse and longitudinal antebasal impressions and with few more impressions on disk. Procoxal cavity closed behind.

Elytron flat or carinate, with large, deep punctures forming striae. Humeral callus well developed. Epipleuron more or less vertical, almost reaching elytral apex.

Metafemur slightly swollen. Metatibia cylindrical, slightly expanded distally; apex oblique. Metatarsus inserted apically. First metatarsal segment short, shorter than following two segments combined.

Key to species of *Sangariola* Jacobson

1. Elytron flat, no visible ridges between striae .... *Sangariola hirashimai*
   Elytron carinate, with visible ridges between striae 2
2. Pronotum and elytron pubescent 3
   Pronotum and elytron glabrous 4
3. Pubescence on elytron longer; antenna wider; only sides of ventral disc at apex of penis membranous .... *Sangariola yuae* sp. nov.
   Pubescence on elytron shorter; antenna narrower; central park of apex of ventral disc at apex of penis membranous .... *Sangariola fortunei*
4. Elytron without elevated ridges between striae; penis slender .... *Sangariola punctatostriata*
   Elytron with elevated ridges between striae 3 and 4, 7 and 8; penis wide .... *Sangariola costata*

***Sangariola hirashimai* Kimoto, 1970**

***Sangariola hirashimai* Kimoto 1970: 213.**

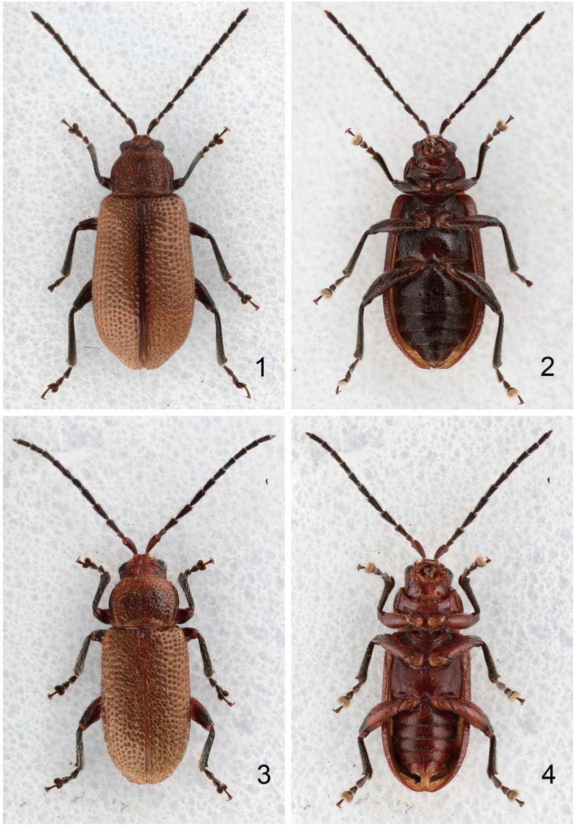
Type Specimens (n = 2). Holotype ♀ (KUEC): "(Taiwan) /Alishan, 2,300 m/Chiayi Hsien (p, w) //9 (h). iv.1965 (p) /Y. Hirashima (p, w) //Japan-U.S./Co-op. Sci./Programme (p, y) //*Sangariola*/*hirashimai*/Kimoto, n. sp. (h, w) //HOLOTYPE (p, r)". Paratype: 1♀ (KMNH): "(Taiwan) /Alishan, 2,300 m/Chiayi Hsien (p, w) //6. (h) vii. 1965 (p) /T. Nakane (p, w) //Japan-U.S./Co-op. Sci./Programme (p, y) //*Sangariola*/*hirashimai*/Kimoto, n. sp. (h, w) //PARATYPE (p, b)".

Specimens Examined (n = 41). TAIWAN: Chiayi: 1♂, Alishan, 12.V.2011, leg. C.-F. Lee (TARI); Hualien: 1♂, Pilu, 31.V.2011, leg. M.-H. Tsou (TARI); Kaoshiung: 1♂, Tengchih, 1.X.2008, leg. C.-T. Yao (TARI); 1♂, 1♀, same locality, 2.III.2009, leg. C.-T. Yao (TARI); 1♀, same locality, 23.III.2009, leg. H. Lee (TARI); 1♂, same locality, 24.III.2009, leg. S.-F. Yu (TARI); 1♀, 8.IV.2011, leg. J.-C. Chen (TARI); 1♂, 1♀, same locality, 6.VII.2013, leg. W.-C. Liao (TARI); Nantou: 1♀, Lienhuachih, 24.XI.2007, leg. M.-H. Tsou (TARI); 2♂♂, same locality, 25.II.2009, leg. Y.-C. Lin (TARI); 1♂, Tatachia, 9.V.2011, leg. C.-F. Lee (TARI); 1♀, Tungpu, 20-22.VI.1980, leg. C. C. Chen (TARI); 1♀, same locality, 16-20.IV.1984, leg. K. C. Chou & C. H. Yang (TARI); 1♂, Tunyuan, 29.IV.1992, leg. W. T. Yang (NMNS); 2♂♂, 1♀, Wushe, 19-22.IV.1983, leg. K. C. Chou & S. P. Huang (TARI); 1♂, same locality, Pingtung: 2♂♂, Lilungshan, 5.XI.2009, leg. J.-C. Chen or M.-H. Tsou (TARI); 1♂, Nanjenhu, 15.III.2010, leg. M.-H. Tsou (TARI); 1♀, same locality, 3.I.2011, leg. J.-C. Chen (TARI); 1♂, Peihulushan, 4.XI.2009, leg. M.-H. Tsou (TARI);

1♂, 1♀, Peitawushan, 22.IX.2012, leg. J.-C. Chen (TARI); 2♀♀, Tahanshan, 12.XI.2009, leg. J.-C. Chen (TARI); 1♂, same locality, 17.IX.2010, leg. J.-C. Chen (TARI); 1♂, 1♀, same locality, 4.X.2010, leg. J.-C. Chen (TARI); 1♀, same locality, 31.X.2011, leg. J.-C. Chen (TARI); 1♀, same locality, 1.X.2012, leg. J.-C. Chen (TARI); 1♂, same locality, 30.VII.2013, leg. B.-X. Guo (TARI); 1♀, same locality, 15.X.2013, leg. Y.-T. Chung (TARI); 1♂, Wanlite, 7-28.II.2010, leg. M.-L. Jeng (TARI); Taitung: 2♂♂, 2♀♀, Litao, 6.X.2010, leg. M.-H. Tsou (BMNH).

Remarks. *Sangariola hirashimai* is characterized by the flat elytron with extremely long pubescence.

Male. Length 4.1–6.5 mm, width 1.7–2.5 mm. General color (Figs. 1–2) dark brown; pronotum and elytron yellowish brown; lateral margins and suture black; some individuals with black stripes reduced and ventral surface brown (Figs. 3–4); antennae and legs black. Body covered with long, scattered pubescence. Antenna filiform (Fig. 5), length ratio of antennomere 3 to 11 about 1.0 : 1.2 : 1.2 : 0.9 : 1.0 : 1.0 : 1.0 : 0.9 : 1.3; ratio of length to width from antennomere 3 to 11 about 2.1 : 2.4 : 2.8 : 2.4 : 2.5 : 2.7 : 2.7 : 2.4 : 4.0. Pronotum flat, without swellings or grooves; with dense and random large punctures. Elytral punctures arranged into 11 longitudinal striae, arrangement of striae 1



Figs. 1–4. Color habitus of *Sangariola hirashimai*. 1. Female, dorsal view; 2. Ditto, ventral view; 3. Male, dorsal view, color variation; 4. Ditto, ventral view, color variation.

to 3 confused; without distinctive ridges between striae. Penis (Figs. 7–8) slender, about 6.0 times longer than wide; abruptly narrowed near apex, apex truncate; parallel sided; slender in lateral view.

Female. Length 4.9–7.1 mm, width 2.2–3.3 mm. Similar to male. Antenna filiform (Fig. 6), length ratio of antennomere 3 to 11 about 1.0 : 1.1 : 1.1 : 1.0 : 1.0 : 1.0 : 1.0 : 1.0 : 1.4; ratio of length to width from antennomere 3 to 11 about 2.1 : 3.1 : 3.1 : 2.8 : 2.9 : 2.8 : 2.5 : 2.6 : 3.6. Gonocoxae (Fig. 10) curved at middle, each gonocoxa with nine setae from apical 1/4 to apex. Sternite VIII (Fig. 9) weakly sclerotized; with dense short setae along apex, disc scattered with less longer setae; spiculum slender, base slightly widened. Spermathecal receptaculum (Fig. 11) slightly swollen, straight; pump strongly curved; spermathecal duct long.

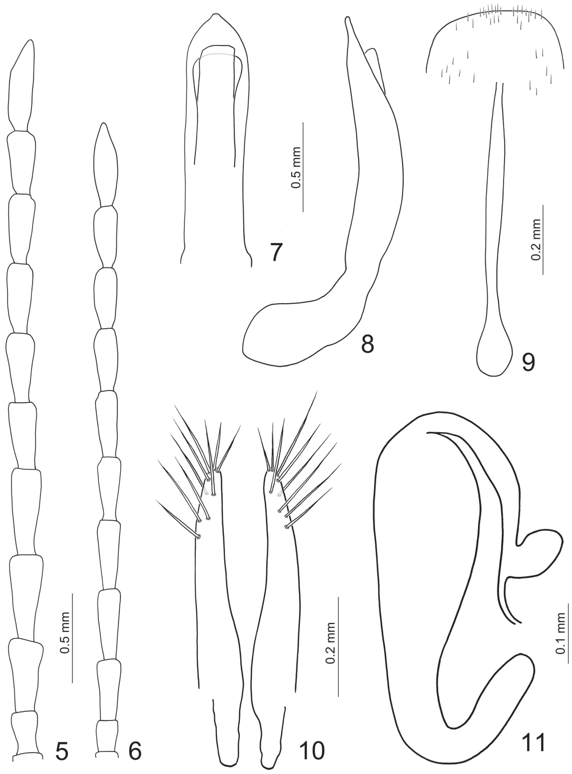
Host plants. Smilacaceae: *Smilax lanceifolia* Roxb. and *Smilax luei* T. Koyama.

Distribution. Endemic to Taiwan. *Sangariola hirashimai* is a common and widespread species in Taiwan (Fig. 12). Most populations inhabit lower elevation habitats (0–1,500 m).

Sangariola yuae **Lee, sp. nov.**

Sangariola fortuntei: Chûjô 1935b: 394 (misidentification); Kimoto 1970: 214; Kimoto 1989: 261.

Type Specimens Examined (n = 21). Holotype ♂ (TARI): Taiwan: Kaoshiung: Tengchih, 26.V.2009, leg. C.-F. Lee. Paratypes:

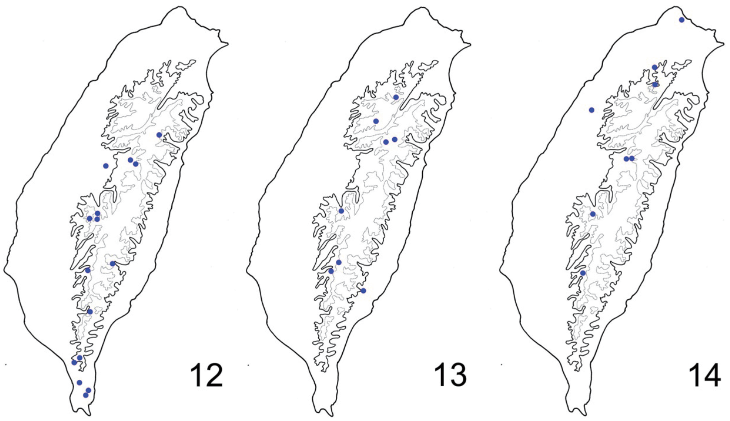


Figs. 5–11. *Sangariola hirashimai*. 5. Antenna, male; 6. Antenna, female; 7. Penis, dorsal view; 8. Penis, lateral view; 9. Sternite VIII; 10. Gonocoxae; 11. Spermatheca.

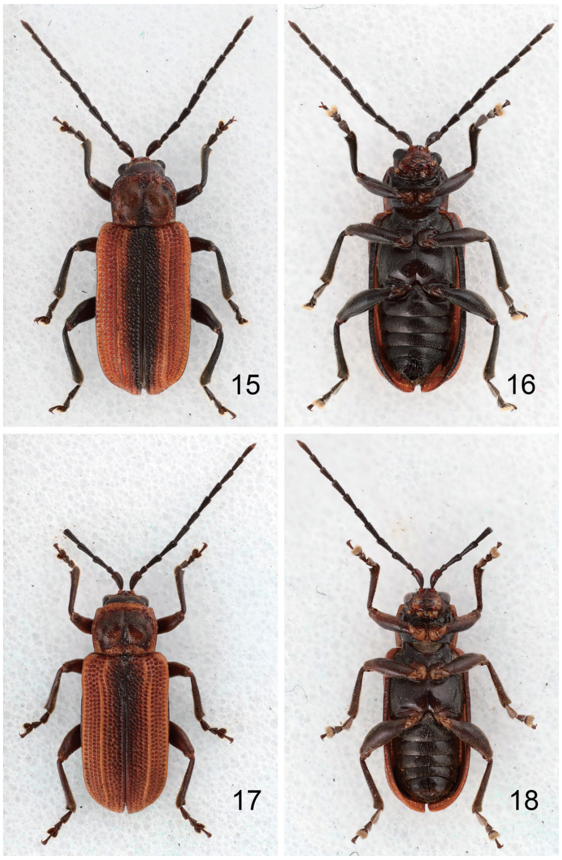
2♀♀ (TARI), same data as holotype; 1♂ (TARI), same locality, 24.V.2005, leg. C.-F. Lee; 2♀♀ (BMNH), same locality, 2-5.VI.2008, leg. C.-F. Lee; 2♂♂, 1♀ (TARI), same locality, 25.V.2009, leg. S.-F. Yu; 1♀ (TARI), same locality, 26.V.2009, leg. H. Lee; 1♂ (TARI), Chungchihkuan, 15.IV.-10.X.2012, leg. L.-P. Hsu; Hsinchu: 1♂, 2♀♀ (TARI), Mamei, 4.V.2008, leg. S.-F. Yu; Hualien: 1♂ (TARI), Tayuling, 9–16.VI.1980, leg. K. S. Lin & B. H. Chen; Nantou: 1♂ (TARI), Hsitou, 6.V.2009, leg. C.-F. Lee; 1♂ (TARI), Meifeng, 7–9.V.1981, leg. K. S. Lin & S. C. Lin; Taichung: 1♂ (TARI), Anmashan, 21.IV.2010, leg. C.-F. Lee; 1♀ (TARI), Tahsuehshan, 23.VII.2011, leg. J.-C. Chen; 1♂ (TARI), Wuwoweishan, 5.VI.2011, leg. J.-C. Chen; Taitung: 1♂ (TARI), Yanping, 17.VI.2010, leg. J.-C. Chen.

Remarks. This new species is similar to *Sangariola fortunei* with dense pubescence and the elevated interstices 4 and 8 on elytra. It can be distinguished from the latter by the longer pubescence on the elytra, the wider antenna, the membranous sides of ventral surface at apex of the penis, and the wider penis in lateral view.

Male. Length 6.1–6.3 mm, width 2.4–2.6 mm. General color (Figs. 15–16) blackish brown; pronotum and elytron reddish brown; elytra with a median longitudinal black stripe from suture to stria 3, elytral epimeron black. Body covered with dense pubescence; disc of head and pronotum with micro-reticulation. Antenna filiform (Fig. 19), length ratio of antennomere 3 to 11 about 1.0 : 1.3 : 1.2 : 1.1 : 1.1 : 1.1 : 1.1 : 1.1 : 1.3; ratio of length to width from antennomere 3 to 11 about 2.0 : 2.8 : 2.8 : 2.5 : 2.6 : 2.8 : 2.8 : 3.0 : 3.9. Pronotum with two large strongly elevated, subrounded swellings occupying much of disc, separated medially by a narrow groove; sometimes with lateral groove dividing into two swellings; disc with large punctures but reduced on swellings. Elytral punctures arranged into 10 longitudinal striae, arrangement of striae 1 to 3 not a little confused; with interstices 4 and 8 strongly elevated. Penis (Figs. 21–22) wide, about 4.3 times longer than wide; abruptly narrowed near apex, apex truncate; parallel sided; strongly widened



Figs. 12–14. Distribution map of *Sangariola* species, solid line: 1,000 m, broken line: 2,000 m. 12. *S. hirashimai*; 13. *S. yuae* sp. nov; 14. *S. costata*.



Figs. 15–18. Color habitus of *Sangariola* species. 15. *S. yuae* sp. nov., male, dorsal view; 16. Ditto, ventral view; 17. *S. fortunei*, male, dorsal view; 18. Ditto, ventral view.

at middle in lateral view; ventral disc with sides of apical 1/3 membranous.

Female. Length 5.5–6.1 mm, width 2.1–2.5 mm. Similar to male. Antenna filiform (Fig. 20), length ratio of antennomere 3 to 11 about 1.0 : 1.2 : 1.3 : 1.2 : 1.2 : 1.1 : 1.1 : 1.1 : 1.3; ratio of length to width from antennomere 3 to 11 about 2.2 : 2.6 : 3.2 : 2.7 : 2.8 : 2.8 : 2.9 : 2.9 : 3.7. Gonocoxae (Fig. 24) curved inwards at apical 1/4, each gonocoxa with nine setae from apical 1/4 to apex. Sternite VIII (Fig. 23) weakly sclerotized; with dense short setae along apex, disc scattered with less longer setae; spiculum slender, base strongly widened. Spermathecal receptaculum (Fig. 25) slightly swollen and curved; pump strongly curved; spermathecal duct long.

Color variation. In some individuals the pronotum is dark brown but the rest of the body is reddish brown. Reddish brown areas on the head are extremely variable.

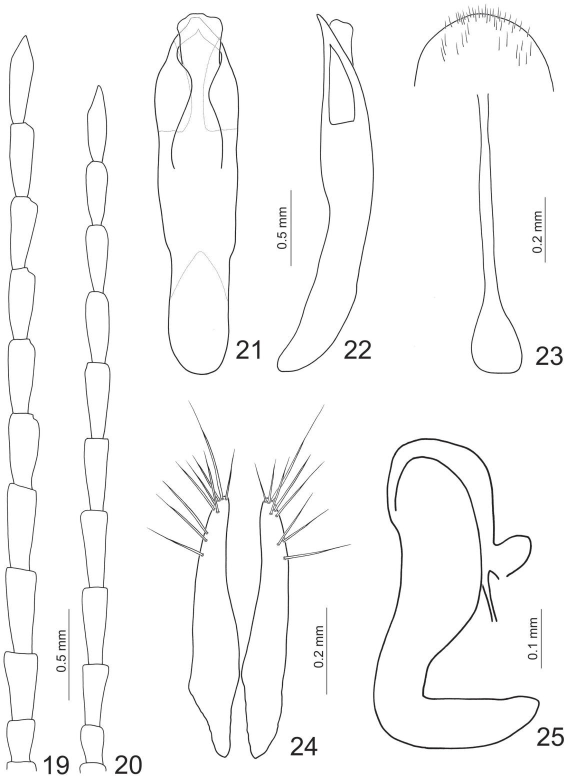
Host plant. Smilacaceae: *Smilax lanceifolia* Roxb.

Distribution. Endemic to Taiwan. This species has a scattered distribution in Taiwan. Most populations inhabit mountain areas (1,000–2,500 m) (Fig. 13).

***Sangariola fortunei* (Baly 1888)**

Charidea fortunei Baly 1888: 158.

Allophyla auora Weise 1889: 626; Weise 1905:



Figs. 19–25. *Sangariola yuae* sp. nov. 19. Antenna, male; 20. Antenna, female; 21. Penis, dorsal view; 22. Penis, lateral view; 23. Sternite VIII; 24. Gonocoxae; 25. Spermatheca.

188 (as synonym of *fortunei*).

Charidea bicostata Pic 1928: 32; Döberl 2010: 558 (as synonym of *fortunei*).

Sangariola fortunei: Chen 1933: 230.

Charidea nigrosuturalis Pic 1937: 176; Gressitt and Kimoto 1963: 755 (as synonym of *fortunei*).

Charidea nigrosuturalis var. *obliterata* Pic 1937: 176. new synonym

Charidea pieli Pic 1937: 175; Döberl 2010: 558 (as synonym of *fortunei*).

Charidea bicostata var. *atriceps* Pic 1938: 18; Gressitt and Kimoto 1963: 755 (as synonym of *fortunei*).

Sangariola fortunei var. *unicoloripennis* Chûjô 1941: 173 (Korea).

Type Specimens (n = 8). *Charidea fortunei*: Lectotype ♀ (BMNH), here designated, pinned and mounted on small card, labeled: "Type (p, circle label with red border) //N. China (h, w) //Journ. Linn. Soc /XX p. 158 (h, w) //*Charidea/fortunei*, Baly/Type (h, w; on back of previous label) //Baly Coll. (p, w) //SYN-/TYPE (p, circle label with blue border) //BMNH(E) /#1024880 (p, w)". Paratype: 1 (sex undetermined), labeled: "N. China (h, w) // SYN-/TYPE (p, circle label with blue border) //Baly Coll. (p, w)".

Allophyla auora: Unavailable for study. They are not presented in the NHRS nor in the Zoogisches Institut und Zoologisches Museum der Universität Hamburg.

Charidea bicostata: Holotype ♂ (MNHN), mounted on triangular card, labeled:

"Chapa (h, w) //Ongles/appendiculès/près genre Laboissierea (h, w) //type (h, w) //*Charidea*/*bicostata* n sp (h, w) //Muséum Paris/1958/Coll. Pic (p, y') //TYPE (p, r) //HOLOTYPE (p, r]) //HOLOTYPE/*Charidea*/*bicostata* Pic, 1928 (p, w) //MNHN/EC4381 (p, w)".

Charidea bicostata var. *atriceps*: Holotype ♂ (NHRS), pinned and labeled: "China/*Aders* (p, w) //Prov./Anhui (p, w) //*bicostaca* Pic (h, w) //v. *atriceps* mihi (h, w) //NHRS-JLKB/000022855 (p, w)".

Charidea nigrosuturalis: Lectotype ♂ (MNHN), here designated, pinned and labeled: "T'iemo (p) u (h) Shan (p) /Musée Heude (p, y) //*P*. (h) /462 (p, gray label) //17.6.36 (h) /O. PIEL, coll. (p, w) //*Charidea*/*nigrosuturalis*/nm (h, w) //Muséum Paris/1958/Coll. Pic (p, y') //SYNTYPE (p, r) //SYNTYPE/*Charidea*/*nigrosuturalis* Pic, 1937 (p, w) //MNHN/EC4384 (p, w)". Paralectotype: 1 ♀ (MNHN): "Mokan Shan/Musée Heude (p, y) //P. (h) /462 (p, gray label) //30.4.36 (h) /O. PIEL, coll. (p, w) //Muséum Paris/1958 /Coll. Pic (p, y') //SYNTYPE (p, r) //SYNTYPE/*Charidea*/*nigrosuturalis* Pic, 1937 (p, w) //MNHN/EC4385 (p, w)".

Charidea nigrosuturalis var. *obliterata*: Holotype ♀ (MNHN), pinned and labeled: "Mokan Shan/Musée Heude (p, y) //*P*. (h) /463 (p, gray label) //13.3.36 (h) /O. PIEL, coll. (p, w) //v. *obliterata*/mihi (h, w) //Muséum Paris/1958/Coll. Pic (p, y') //SYNTYPE (p, r) //SYNTYPE/*Charidea*/*nigrosuturalis*/var. *obliterata* Pic, 1937 (p, w) //MNHN/EC4386 (p, w)".

Charidea pieli: Holotype ♂ (MNHN), pinned and labeled: "Mokan Shan/Musée Heude (p, y) //*P*. (h) /461 (p, gray label) //6.5.36 (h) /O. PIEL, coll. (p, w) //Muséum Paris/1958/Coll. Pic (p, y') //*Charidea*/*pieli* nm (h, w) //HOLOTYPE (p, r) //HOLOTYPE/*Charidea*/*pieli* Pic, 1937 (p, w) //MNHN/EC4383 (p, w)".

Sangariola fortunei var. *unicoloripennis*: Holotype ♂ (TARI): "Kôryô, Yôsyû-Gun/Keiki-Dô (h) /COREA (p) /16.V.1937 (h) /COL. (p) T. KusanagiI (h, w) //Holotype (letters faded, circle label) //*Sangariola*/*fortunei* Baly/var. *unicoloripennis*/Chûjô (h) /DET. M. CHUJO (p, w) //2235 (p, w)".

Specimens Examined (n = 22). CHINA: Fujian: 1♀, Chanting, Niuling, 21.IV.1941, leg. T. C. Maa (CAS); 12 exs., Foochoo, VI.1935, leg. M. S. Yang (BMNH); 1♂, Shaowu, Tachulan, 12.V.1942, leg. T. Maa (CAS); 1♂, same locality, 6.V.1945, leg. T. C. Maa (BPBM); 1♂, 3♀♀, Shaowu, Shuipeikai, 11.IV.1943, leg. T. C. Maa (CAS); 1♂, same locality, V.1944, leg. T. C. Maa (CAS); Zhejiang: 1♀, 18.V.1923, leg. Van Dyke (CAS); 1♂, Yian City, Changhua, 16.V.2010, leg. Y.-F. Hsu (TARI).

Remarks. *Sangariola fortunei* is similar to S. *yuae* sp. nov. with dense pubescence and the elevated interstices 4 and 8 on elytra, but can be distinguished from the latter by the shorter pubescence on the elytra, the narrower antenna, and the membranous central part of the ventral surface at apex of penis.

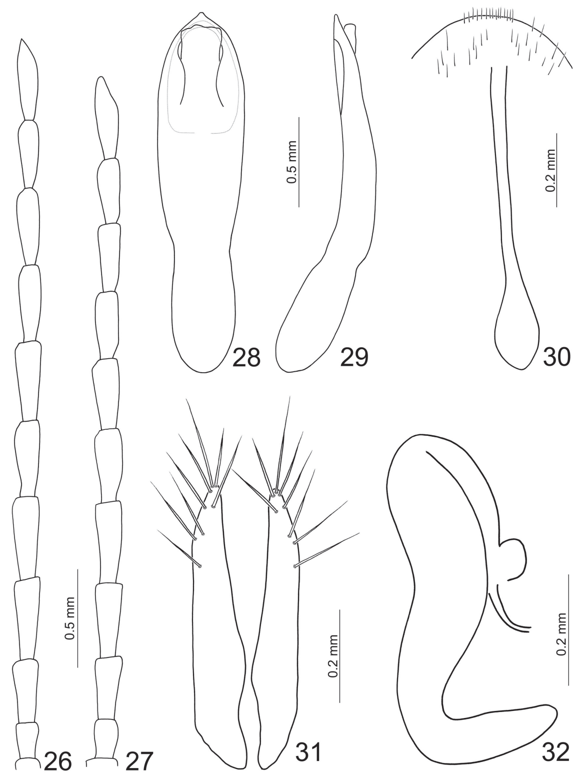
Male. Length 5.0–6.3 mm, width 2.2–2.5 mm. General color (Figs. 17–18) dark brown; pronotum and elytron reddish brown; elytra with a median longitudinal black stripe from suture to stria 3, elytral epimeron black. Body covered with dense pubescence; disc of head and pronotum with micro-reticulation. Antenna filiform (Fig. 26), length ratio of antennomere 3 to 11 about 1.0 : 1.3 : 1.3 : 1.2 : 1.2 : 1.2 : 1.2 : 1.1 : 1.3; ratio of length to width from

antennomere 3 to 11 about 2.5 : 3.1 : 3.1 : 3.1 : 3.1 : 3.2 : 3.4 : 3.3 : 3.9. Pronotum with two large strongly elevated, rounded swellings occupying much of disc, separated medially by a narrow groove; sometimes with lateral groove dividing into two swellings; disc with large punctures but reduced on swellings. Elytral punctures arranged into 10 longitudinal striae, arrangement of striae 1 to 3 a little confused; with interstices 4 and 8 strongly elevated. Penis (Figs. 28–29) wide, about 4.1 times longer than wide; abruptly narrowed near apex, apex truncate; widest at apical 1/3; slender in lateral view; ventral disc with apical 1/3 membranous.

Female. Length 5.3–5.9 mm, width 2.3–2.4 mm. Similar to male. Antenna filiform (Fig. 27), length ratio of antennomere 3 to 11 about 1.0 : 1.3 : 1.3 : 1.2 : 1.2 : 1.1 : 1.1 : 1.0 : 1.3; ratio of length to width from antennomere 3 to 11 about 2.3 : 3.0 : 3.0 : 3.0 : 2.9 : 3.1 : 3.0 : 3.0 : 3.9. Gonocoxae (Fig. 31) straight, each gonocoxa with eight setae from apical 1/4 to apex. Sternite VIII (Fig. 30) weakly sclerotized; with one row of short setae along apex, disc scattered with less longer setae; spiculum slender, base strongly widened. Spermathecal receptaculum (Fig. 32) slightly swollen and curved; pump strongly curved; spermathecal duct long.

Host plants. Smilacaceae: *Smilax* spp.; Liliaceae: *Lilium* spp.; Araceae: *Symplocarpus* spp. (Yu 1996).

Distribution. China (Fujian, Guandong, Guizhou, Hunan, Yunnan, Zhejiang), Korea, Vietnam.



Figs. 26–32. *Sangariola fortunei*. 26. Antenna, male; 27. Antenna, female; 28. Penis, dorsal view; 29. Penis, lateral view; 30. Sternite VIII; 31. Gonocoxae; 32. Spermatheca.

Notes. No papers officially treated *Charidea bicostata* Pic, 1928 and *C. pieli* Pic, 1937 as synonyms of *Sangariola fortunei* (Baly 1888), but Döberl (2010) listed them as junior synonyms. Gressitt and Kimoto (1963) proposed *Charidea bicostata* var. *atriceps* Pic, 1938 as a junior synonym of *S. fortunei* but they also indicated that the original description of *C. bicostata* was not traced. Actually, *C. bicostata* was published by Pic (1928).

***Sangariola punctatostriata* (Motschulsky 1861)**

Galleruca (sic!) *punctatostriata* Motschulsky 1861: 25.

Galeruca (*Adimonia*) *multicostata* Jacoby 1885: 746; Chûjô 1935a: 86 (as synonym of *punctatostriata*).

Charidea regularis Pic 1928: 31; Chûjô and Kimoto 1961: 191 (as synonym of *multicostata*).

Sangariola punctatostriata: Chûjô 1935a: 86 (Okinawa island).

Sangariola punctatostriata aequicostata Chûjô 1938: 10; Chûjô and Kimoto 1961: 191 (as synonym of *punctatostriata*).

Type Specimens (n = 15). *Galleruca* (sic!) *punctatostriata*: Lectotype and one paralectotype (sex undetermined) (ZUMU), designated by Medvedev (2006), mounted on card and with same pin, labeled: "*Galleruca*/*punctatostri*/*-ata* Motsch/Japan (h, w)"; 1♀ (BMNH), pinned on small card, labeled: "Jap (h) //*Galleruca*/*punctatostriata* Motsch/Japan (h, w) //*Charidea* (h, w) //Baly coll (p, w)".

Galeruca (*Adimonia*) *multicostata*: Lectotype (sex undetermined) (BMNH), mounted on card, here designated and labeled: "(back of card mounted with lectotype) *Lilium*/*auratum*/May (18) 80 (with pencil) //Jacoby Coll./1909–28a (p, w)". Paralectotypes: 1 (sex undetermined) (BMNH): "Suyama./20.IV-22.IV (p, w; posterior half cut off) //Japan./G. Lewis. /1910–320. (p, w) //on *Lilium auratum*/& *L. lancifolium* (h, w) //SYN- /TYPE (p, circle label with blue border)"; 1 (sex undetermined) (BMNH): "Japan./G. Lewis./1910–320. (p, w) //SYN-/TYPE (p, circle label with blue border)"; 2 (sex undetermined) (BMNH): "Japan./G. Lewis./1910–320. (p, w)"; 1 (sex undetermined) (BMNH), same but with additional label: "IX.8, small beetle (written in Chinese)"; 2 (sex undetermined) (MCZC): "Japan/Lewis (h, w) //Type./Sp. figured. (p, w) //1st Jacoby/Coll. (p, w) //*A. multicostata* (h, purple label) //Type (p) /18208 (h, red label)"; 1 (sex undetermined) (TARI): "Ichiuchi (h) /JAPAN (p) /30.IV.1881 (h) /Col. G. LEWIS (p, w) //CO/Type (p, yellow letter, circle label with yellow border) //Cotype of *Galeruca*/*multicostata* Jacoby (h, w) //*Sangariola*/*punctatostriata*/Motschulsky (h) /DET. M. CHUJO (p) //1421 (p, w)"; 1 (sex undetermined) (TARI): "Ichiuchi (h) /JAPAN (p) /30.IV.1881 (h) /Col. G. Lewis (p, w) //CO/Type (p, yellow letter, circle label with yellow border) //*Galeruca*/*multicostata* Jac. (h) /Det. T. Shiraki (p, w) //*Sangariola*/*punctatostriata*/Motschulsky (h) /DET. M. CHUJO (p, w)".

Charidea regularis: Holotype ♂ (MNHN): "Kioto/Japon (h, w) //type (h, w) //*Charidea*/*regularis* Pic (h, w) //Muséum Paris /1958/Coll. Pic (p, y') //SYNTYPE (p, r) //SYNTYPE/*Charidea*/*regularis* Pic, 1928 (p, w) //MNHN/EC4382 (p, w)".

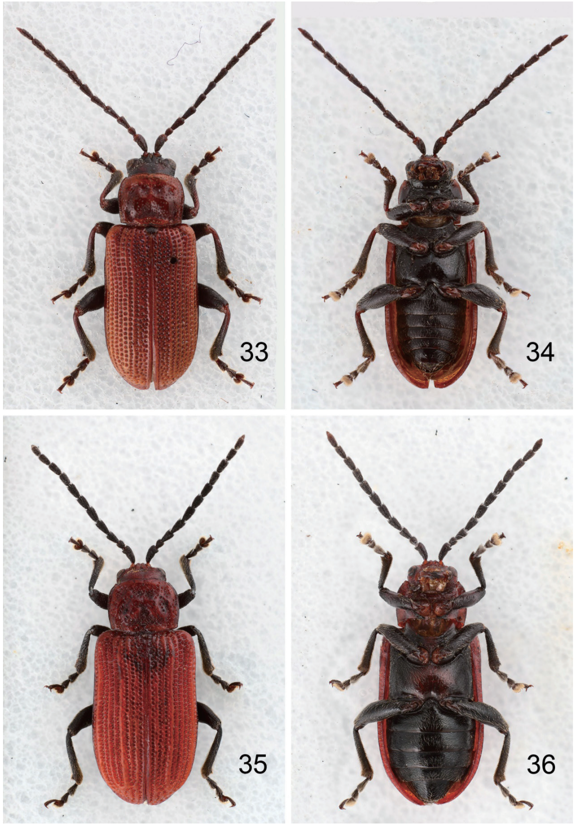
Sangariola punctatostriata aequicostata: Holotype ♀ (TARI) (automatically fixed by monotype): "Sobosan (h) /KYÛSHÛ,

JAPAN (h) /4.VII.1937 (h) /Coll. S. Issiki (h, w) //Typus (h) //*Sangariola* (h) / *punctatostriata* (h) Motschulsky subsp. (h) /*aequicostata* CHUJO (h) /DET. M. Chûjô (p, pink label) //2927 (p, w)".

Specimens Examined (n = 12). JAPAN: Sikoku: 3♂♂, 4♀♀, Kooti-Ken, 26.III.1934-16.VI.1935, leg. I. Okubo (TARI); Kyushu: 1♂, Mt. Inunaki, Fukuoka-Ken, 5.V.1940, leg. S. Nishiguchi (TARI); Okinawa: 1♂, V.1925, leg. S. Sakaguchi (TARI); Hokkaido: 1♀, Dôyako, III.1928, leg. Y. Miwa (TARI); RUSSIA: 1♂, Saghalien, leg. K. Tamanuki (BMNH).

Remarks. *Sangariola punctatostriata* and *S. costata* are characterized by lacking pubescence on the pronotum and elytra. *S. punctatostriata* may be distinguished from the latter by more (11) elytral striae (10 striae in *S. costata*), without elevated ridges between striae on elytron (with elevated ridges between striae 3 and 4, 7 and 8 in *S. costata*), and confused striae 1 to 3 (all striae regularly arranged in *S. costata*).

Male. Length 5.1–6.1 mm, width 2.3–2.8 mm. General color (Figs. 33–34) dark brown to blackish brown; but pronotum and elytron reddish brown. Body covered with dense pubescence except pronotum and elytron; disc of head and pronotum with micro-

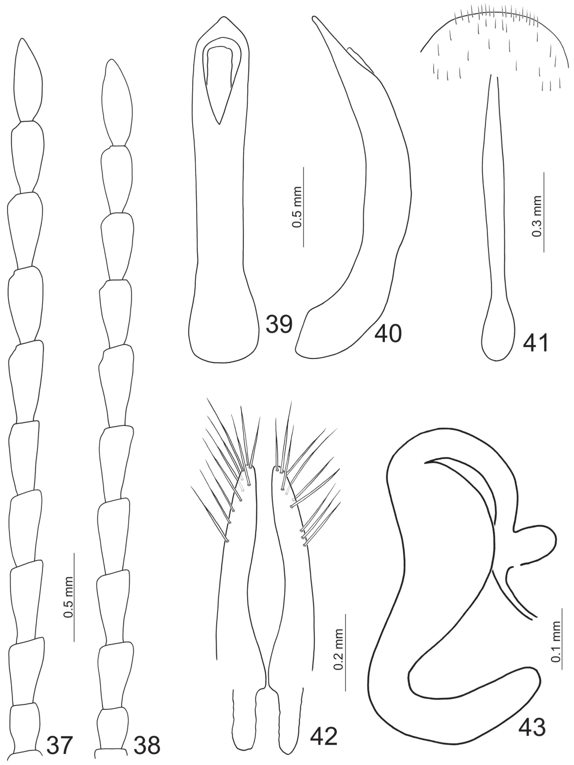


Figs. 33–36. Color habitus of *Sangariola* species. 33. *S. punctatostriata*, male, dorsal view; 34. Ditto, ventral view; 35. *S. costata*, female, dorsal view; 36. Ditto, ventral view.

reticulation. Antenna filiform (Fig. 37), length ratio of antennomere 3 to 11 about 1.0 : 1.3 : 1.1 : 1.1 : 1.3 : 1.2 : 1.2 : 1.2 : 1.4; ratio of length to width from antennomere 3 to 11 about 1.8 : 2.4 : 2.2 : 2.3 : 2.3 : 2.2 : 2.1 : 2.2 : 2.9. Pronotum with dense and large punctures, arrangement of punctures not regular, density greatest near basal and apical margin, with medial and lateral grooves punctate. Elytral punctures arranged into 11 longitudinal striae, arrangement of striae 1 to 3 confused, without elevated interstices; shape extremely variable from 1.6 to 1.9 times longer than wide. Penis (Figs 39–40) extremely slender, about 6.6 times longer than wide; parallel sided, abruptly narrowed near apex, apex narrowly rounded; slender in lateral view, moderately curved at middle.

Female. Length 5.3–6.6 mm, width 2.2–3.3 mm. Similar to male. Antenna filiform (Fig. 38), length ratio of antennomere 3 to 11 about 1.0 : 1.2 : 1.2 : 1.2 : 1.3 : 1.1 : 1.1 : 1.1 : 1.4; ratio of length to width from antennomere 3 to 11 about 1.8 : 2.2 : 2.2 : 2.2 : 2.3 : 2.0 : 2.0 : 2.0 : 3.5. Gonocoxae (Fig. 42) curved inwards at middle, each gonocoxa with ten setae from apical 1/3 to apex. Sternite VIII (Fig. 41) weakly sclerotized; with dense short setae along apex, disc scattered with less longer setae; spiculum slender, base strongly widened. Spermathecal receptaculum (Fig. 43) slightly swollen and curved; pump strongly curved; spermathecal duct long.

Host plants. Liliaceae: *Lilium cordatum* (Thunb.) Koidz.; *Lilium Leichtlinii* Hook. fil. var. *tigrinum* (Regal) Nicols.; and *Ery-*



Figs. 37–43. *Sangariola punctatostriata*. 37. Antenna, male; 38. Antenna, female; 39. Penis, dorsal view; 40. Penis, lateral view; 41. Sternite VIII; 42. Gonocoxae; 43. Spermatheca.

thronium japonicum Decen. Smilacaceae: *Smilax China* Linn. (Chûjô and Kimoto 1961).

Distribution. Russia, Korea, Japan.

Notes. One specimen bears the holotype label and is deposited at the BMNH. It is labeled: "Type/H. T. (p, circle label with red border) //Japan/G. Lewis/1910–320 (p, w) //Yokohama./20.III.-14.IV.80 (p, w) // *Adimonia*/*multicostata* Jac. (h, purple label)", is excluded from the type series because critical information is missing from the description "Yokohama". *Galleruca* is a wrong spelling for "*Galeruca*" but Motschulsky kept using it.

***Sangariola costata* Chûjô, 1935 stat. recur.**

Sangariola costata Chûjô 1935b: 394; Kimoto 1970: 214 (as synonym of *punctatostriata*).

Sangariola punctatostriata: Kimoto 1970: 214 (misidentification); Kimoto 1987: 191; Kimoto and Chu 1996: 95.

Type Specimens (n = 3). *Sangariola costata*: Lectotype ♂ (TARI), here designated and labeled: "Formosa./Musha (= Wushe, in Nantou county), 1919/V 18 – VI 19,/T. OKUNI, (p, w) //CO/Type (p, yellow letters, circle label) //*Sangariola*/*costata*/ Chûjô (h) /DET. M. CHUJO (p, b) //1661 (p, w)". Paralectotypes: 1♂ (TARI), same as holotype but with "1938"; 1♀ (TARI): "Kappanzan (= Chiaopanshan, in Taoyuan county) (h) /FORMOSA (p) /26.IV.1933 (h) /COL. T. MITONO (p, w)//CO/Type (p, yellow letters, circle label) //*Sangariola*/ *costata*/Chûjô (h) /DET. M. CHUJO (p, blue label) //708 (p, w)"

Specimens Examined (n = 54). Taiwan: Kaoshiung: 1 ex., Chungchihkuan, 16.IV.2012, leg. L.-P. Hsu (TARI); Miaoli. 5 exs., Tahu, 7.IV.2013, leg. D. Lu (BMNH); Nantou: 1♀, Lushan, 27–31. V.1980, leg. K. S. Lin & L. Y. Chou (TARI); 1♀, Tungpu, 20–22.VI.1980, leg. C. C. Chen (TARI); 2♂♂, 1♀, same locality, 28.IV.–2.V.1981, leg. T. Lin & C. J. Lee (TARI); 9♂♂, 2♀♀, same locality, 16–20.IV.1984, leg. K. C. Chou & C. H. Yang (TARI); 3♀♀, Wushe, 19–22. IV.1983, leg. K. C. Chou & S. P. Huang (TARI); 1♂, 1♀, same locality, 7.V.1984, leg. K. C. Chou & C. C. Pan (TARI); 3 exs., Wanfengtsun, 18.IV.2011, leg. W.-T. Liu (TARI); Taipei: 1 ex., Chinshan, 20.III.2010, leg. C.-C. Cheng (TARI); Taoyuan: 5♀♀, Paling, 3–5.V.1983, leg. K. C. Chou & C. C. Pan (TARI); 4 exs., same locality, 21.III.2010, leg. M.-H. Tsou (TARI); 7 exs., Tungyanshan, 10.V.2009, leg. S.-F. Yu or M.-H. Tsou (TARI); 1 ex., same locality, 12.VI.2009, leg. H. Lee (TARI); 5 exs., same locality, 8.IV.2012, leg. M.-H. Tsou (TARI).

Remarks. *Sangariola costata* is similar to *S. punctatostriata*. For diagnosis of both species, see remarks under *S. punctatostriata*.

Male. Length 5.4–6.3 mm, width 2.5–2.8 mm. General color (Figs. 35–36) black; but head, pronotum, prothorax, scutellum, and elytron reddish brown. Body covered with dense pubescence except pronotum and elytron; disc of head and pronotum with micro-reticulation. Antenna filiform (Fig. 44), length ratio of antennomere 3 to 11 about 1.0 : 1.2 : 1.2 : 1.1 : 1.2 : 1.1 : 1.2 : 1.2 : 1.5; ratio of length to width from antennomere 3 to 11 about 1.8 : 2.0 : 2.3 : 2.1 : 2.1 : 2.1 : 2.4 : 2.7 : 3.4. Pronotum with dense and large punctures, arrangement of punctures not regular, most dense near basal and apical margin, with medial and lateral grooves punctate. Elytral punctures

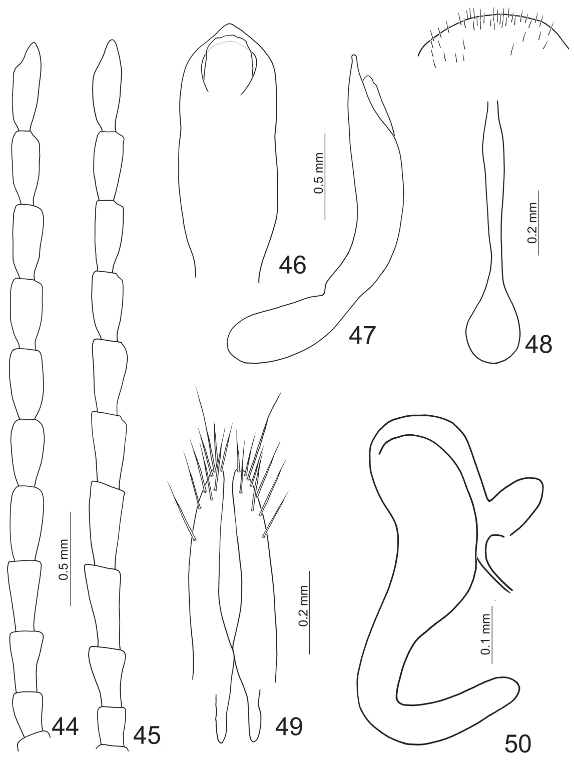
arranged into ten longitudinal striae, with elevated interstices between striae 3 and 4, 7 and 8. Penis (Figs. 46–47) extremely wide, about 3.4 times longer than wide; parallel sided, abruptly narrowed near apex, apex narrowly rounded; wide in lateral view, widest at apical 1/3, strongly curved at middle.

Female. Length 5.1–6.4 mm, width 2.3–3.1 mm. Similar to male. Antenna filiform (Fig. 45), length ratio of antennomere 3 to 11 about 1.0 : 1.4 : 1.4 : 1.2 : 1.3 : 1.2 : 1.2 : 1.2 : 1.3; ratio of length to width from antennomere 3 to 11 about 1.6 : 2.3 : 2.4 : 2.0 : 2.2 : 2.1 : 2.4 : 2.6 : 3.2. Gonocoxae (Fig. 49) curved inwards at middle, each gonocoxa with nine setae from apical 1/4 to apex. Sternite VIII (Fig. 48) weakly sclerotized; with dense short setae along apex, disc scattered with shorter setae; spiculum slender, base strongly widened. Spermathecal receptaculum (Fig. 50) slightly swollen and curved; pump strongly curved; spermathecal duct long.

Host plant. Liliaceae: *Lilium longiflorum* var. *formosanum* Baker.

Distribution. Endemic to Taiwan. It is a widespread species closely associated with its host plant.

Notes. Kimoto (1970) treated *Sangariola costata* as a synonym of *S. punctatostriata* which is widespread in east Asia. Actually, *S. costata* is a distinct species and endemit to Taiwan.



Figs. 44–50. *Sangariola costata*. 44. Antenna, male; 45. Antenna, female; 46. Penis, dorsal view; 47. Penis, lateral view; 48. Sternite VIII; 49. Gonocoxae; 50. Spermatheca.

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細角葉蚤屬的分類修訂 (鞘翅目：金花蟲科：螢金花蟲亞科：葉蚤族) 標本名錄

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摘要

李奇峰。2014。細角葉蚤屬的分類修訂 (鞘翅目：金花蟲科：螢金花蟲亞科：葉蚤族)。台灣農業研究 63(3):188–204。

本文針對細角葉蚤屬 *Sangariola* 做全世界的種類的分類修訂，共有五個種類被視為有效種，其中重新描述 *Sangariola hirashimai* Kimoto, 1970、*Sangariola fortunei* (Baly 1888) 及*Sangariola punctatostriata* (Motschulsky 1861)；而 *Sangariola costata* Chûjô, 1935b 本來為 *S. punctatostriata* (Motschulsky 1861) 的同物異名，現被移出而視為獨立的種類；此外描述一產於台灣的新種：*Sangariola yuae* sp. nov.。*Charidea nigrosuturalis* var. *obliterata* Pic, 1937 為 *S. punctatostriata* (Motschulsky 1861) 新的同物異名。本文並指定下列的種類的選模標本 (lectotype)：*Charidea fortunei* Baly, 1888、*Charidea nigrosuturalis* Pic, 1937、*Galleruca* (sic!) *punctatostriata* Motschulsky, 1861、*Galeruca* (*Adimonia*) *multicostata* Jacoby, 1885及*Sangariola costata* Chûjô, 1935b。

關鍵詞：分類學、金花蟲、百合。

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白芷 Cinnamate 4-hydroxylase 基因之選殖、功能性分析及 8-氫化佛手柑內酯美白物質量產之研究

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摘要

羅淑卿、鍾佩恩。2014。白芷 Cinnamate 4-hydroxylase 基因之選殖、功能性分析及 8-氫化佛手柑內酯美白物質量產之研究。台灣農業研究 63(3):205–215。

Cinnamate 4-hydroxylase (C4H) 為苯基丙烷類代謝路徑 (phenylpropanoids) 之酵素，合成許多植物重要二級代謝產物，包括白芷藥性成分呋喃香豆素 (furanocoumarins)。為探討白芷 *C4H* 基因及酵素特性，由其它物種 *C4H* 高保守性區域序列中，設計退化性引子，從白芷根中選殖出單一的 *AdC4H* cDNA 部分片段。利用 5'- 與 3'-RACE-PCR 對此白芷 *AdC4H* 片段進行聚合酶連鎖增幅反應以取得全長 cDNA 序列 (accession no. KJ453107)。此 *AdC4H* cDNA 之可編譯區含 1,518 個核苷酸，可轉譯為 505 個胺基酸，分子量預估為 57.9 kDa。AdC4H 經胺基酸序列分析比對顯示和其它植物 C4H 有 74% 以上的相同度，具 cytochrome P450 特有的 A–F 保守性區域及 6 個受質辨識區。將 *AdC4H* 可編譯區 cDNA 建構在 pYES2/NT 載體，另外選殖阿拉伯芥 cytochrome P450 reductase *ATR1* cDNA 全長並建構於 pYES3/CT 載體，以缺乏色胺酸及尿嘧啶之培養基篩選，取得併存二載體 (pYES-C4H 及 pYES3-ATR1) 之轉形酵母菌 (*Saccharomyces cerevisiae*)，大量表現融合蛋白質後，進一步純化融合蛋白以探討酵素特性。酵素生化特性研究顯示 AdC4H 於 25℃、pH 6.0 之磷酸鉀緩衝液具最佳活性。AdC4H 在 ATR1 存在下，催化 cinnamate 羥基化效率明顯增加，產物 ρ-coumaric acid 含量由偵測不到提升至 62.9 μM。具備二載體之轉形酵母菌亦可催化 bergapten 為 8-hydroxybergapten，培養液具抗酪胺酸酶活性，IC_{50} 為 1.35 mg mL^{-1}。本研究證實 AdC4H 屬 cytochrome P450 第二群家族成員，可能為膜蛋白，酵素活性在不同物種之還原酶存在下依然可以顯現。

關鍵詞：白芷、Cinnamate 4-hydroxylase、細胞酵素 P450。

前言

Cinnamate 4-hydroxylase (C4H)，催化 *trans*-cinnamic acid 之 *para*-hydroxylation 生成 *ρ*-coumaric acid (supplemental figure)，為 phenylpropanoid 代謝路徑的第二個酵素。C4H 和 PAL (phenylalanine ammonia-lyase) 及 4CL (4-coumarate CoA ligase) 三酵素組成代謝路徑核心反應之重要酵素，合成許多植物生長所需的二級代謝物質，如木質素 (Ehlting *et al*. 2006)。這三酵素在生合成反應中為連動的，當植物受到外界刺激誘導時，可同時增加活性，其中以 C4H 為主控角色 (Mizutani *et al*. 1997)。C4H 所合成的重要二級代謝物質除木質素外，亦合成其它對人體健康有益的物質，如色素 (類黃酮，flavonoids)、紫外線保護劑 (香豆素，coumarin's) 及植物防禦物質 (異黃酮，isoflavonoids)。C4H 屬 cytochrome P450-dependent enzymes (Cyt P450) 之一員 (Bak *et al*. 1998)。

許多文獻亦稱 Cyt P450 為 CYPs，Nel-

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son *et al*. (2004) 依據胺基酸序列的相似度將 CYPs 區分為數個族 (family) 及亞族 (subfamily)。C4H 歸屬於 CYP73 家族 (Ehlting *et al*. 2006)。Cyt P450 酵素活性可因植物受傷、光線或 UV 照射、金屬離子 (鎂或錳) 或病菌侵害而使 mRNA 表現量改變 (Teutsch *et al*. 1993; Chapple 1998)。Cyt P450 除了合成植物體許多特有物質外，亦參與生物體解毒功能，如代謝固醇類、xenobiotics 和除草劑 (Singh *et al*. 2009)。因此，Cyt P450 對於保護植物機制上扮演重要角色 (Ehlting *et al*. 2006)，決定生物體對藥物之耐受性或除草劑之代謝能力，與醫藥業及農業關係極大。

藉由操控 C4H 可滿足人類商業化之需求，使 C4H 基因大量表現，可以改變植物的代謝路徑，讓植物大量生產對人類有經濟價值的植物二級代謝產物類黃酮 (Yan *et al*. 2005)。相反的，弱化 C4H 基因以降低木質素之生合成，可降低造紙工業的成本 (Baucher *et al*. 2003)，同時作為生質能源之利用 (Hisano *et al*. 2009)，對畜牧業來說，也可使飼料較易消化 (Hu *et al*. 1999)。

白芷之藥性成分為香豆素，由 phenylpropanoid 代謝路徑所合成。本研究擬選殖出白芷 C4H 基因 (*AdC4H*)，希望能對此基因特性有所了解，並轉殖此基因至酵母菌生產白芷美白物質 8-hydroxybergapten (8-HB) (Piao *et al*. 2004)，期可改善白芷藥性物質之量產，提升白芷之經濟價值。

材料與方法

專一性片段及全長 cDNA 之選殖

白芷試驗藥材、5'-rapid amplification of cDNA ends (5'-RACE) cDNA 及 3'-RACE cDNA 之取得及基因選殖方法參考 Lo *et al*. (2012)。參考已發表之 *C4H* 序列 (NCBI, http://www.ncbi.nlm.nih.gov/)，在具高保守性的區域中設計一退化性引子 (degenerate primer) 5'-GGTC (A/T/C) AT (C/T) GA (A/G) TGGGG (A/C) (A/G) TTGC (A/G) GA-3' (P1) 對 3'-RACE cDNA 進行專一性片段選殖。後續 cDNA 全長之選殖則以 P2 (5'-CCCCAAGCACAGTGTCCATCTCATT-3') 及 P3 (5'-GCCCAACAGGGCCTTCTCCAACAACA-3') 二引子對 5'-RACE cDNA 進行專一性片段選殖。選殖方法依照 SMART^{TM} RACE cDNA amplificationkit 操作手冊進行 (CLONTECH)。*ATR1* (*Arabidopsis thaliana NADPH Cyt P450 reductase 1*) 全長序列片段之選殖則以 ATR1-F (5'-CCGGAATTCATGACTTCTGCTTTGTAT-3') 及 ATR1-R (5'-GCGGCCGCCAGACATCTCTGAGGTAT-3') 對阿拉伯芥 Columbia wild type cDNA 釣取 *ATR1* 全長 (Urban *et al*.1997)。

基因序列特性分析

序列比對利用 NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi)。預測訊息胜肽 (signal peptide) 及可能切位則利用 Kyte-Doolittle Hydropathy Profile 軟體 (hydrophathy plot) 與 ExPASy Proteomics tools 的 signal P 軟體。蛋白質預測分子量及等電點以 Vector NTI Suite 8 軟體 (Informax, USA) 進行。

AdC4H 融合蛋白的表現與純化

表現載體之建構與轉形：以全長 AdC4H cDNA 作為模版。以 AdC4H-F (5'-CGCGGATCCGATGGATTTTCTGTTGTT-3'；底線為 *Bam*HI 限制酶切位) 及 AdC4H-R (5'-TGCTCTAGATTATGATCTTGGCTTGCA-3'；底線為 *Xba*I 限制酶切位) 兩引子進行 PCR 增幅，選殖 1,569 鹼基對之片段。將其接合至 *Bam*HI/*Xba*I 截切之 pYES2/NT (B) 載體，所得載體命名為 pYES-C4H。另建構 pYES3-ATR1 載體，利用阿拉伯芥 ATR1 cDNA，經 *Eco*RI 及 *Not*I 兩個限制酵素剪切後，接合至 *Eco*RI/*Not*I 截切之 pYES3/CT 載體，將建構之載體命名為 pYES3-ATR1。將 pYES-C4H 及 pYES3-ATR1 二載體同時轉形至酵母菌 INVSc1 勝任細胞，轉形、培養及誘導方法參照 Invitrogen 公司所附贈使用說明書。為篩選出併存二載體的轉形酵母菌，選用缺乏 tryptophan 及 uracil 之 SC 固態培養基 [0.17% yeast nitrogen base、0.5% ammonium sulfate、0.115% HSM (adenine、arginine、cysteine、leucine、

lysine、threonine：aspartic acid、histidine、isoleucine、methionine、phenylalanine、proline、serine、tyrosine、valine = 2 : 1 w/w)、2% agar、10% glucose]，30℃培養 3 d。誘導後之菌體經離心收集，進行下述 *in vivo* 受質轉化能力檢測及融合酵素活性檢測，或破菌萃取融合酵素進行西方墨跡法檢測 AdC4H 蛋白質產出情形 (Lo *et al*. 2012)。

in vivo 受質轉化能力檢測：取上述經 galactose 誘導之菌體，以含 10% galactose 及 100 μM *trans*-cinnamic acid 的 SC 液體培養基回溶，30℃培養 20 h。所得菌液，添加 ethyl acetate 萃取產物 ρ-coumaric acid，以 UPLC 進行定量分析。

融合酵素 AdC4H 活性檢測：取上述經 galactose 誘導之菌體，參照 Pompon *et al*. (1996) 方法萃取酵素，取得 20 μg 濃縮酵素，加入 200 μL 之 200 mM phosphate buffer (pH 4.5–8.5) 內含 0.5 mM *trans*-cinnamic acid 及 1.2 mM NADPH，於 25℃反應 2 h，再加入 ethyl acetate 終止反應並萃取產物。將 ethyl acetate 有機溶劑層取出後蒸發，再以甲醇回溶過濾，進行產物 (ρ-coumaric acid) 濃度分析定量。

西方墨跡法：蛋白質萃取、濃度檢測、蛋白質電泳 SDS-PAGE (SDS-Polyacrylamide Gel Electrophoresis) 及轉漬方法依照 Lo *et al*. (2012)。免疫抗體反應使用 anti-His tag 抗體進行偵測。

美白發酵液產製與抑制酪胺酸酶活性能力檢測

將經 galactose 誘導之菌體以含 10% galactose 及 100 μM bergapten 的 SC 液體培養基回溶，30℃培養 20 h。所得菌液，添加 ethyl acetate 萃取產物，將 ethyl acetate 有機溶劑層取出蒸乾後，以甲醇回溶過濾，利用超高壓液相層析儀 (Ultra Performance Liquid Chromatography; UPLC) 分析 bergatpen 與 8-HB 產物濃度。美白能力檢測以 Tyrosinase Assay 之方法 (Piao *et al*. 2004) 稍加以修改。將上述以甲醇回溶之物質，以 80℃水浴蒸乾，再加入 5% DMSO 回溶。取 40 μL，加入 120 μL 之 0.3 mg mL^{-1} tyrosine (溶於 66.7 mM phosphate buffer, pH6.8)，混合均勻後，再加 40 μL tyrosinase solution (87.5 units mL^{-1}) 混合反應後，立即測定 475 nm 吸光值，再於 37℃靜置反應 30 min，再次測定 475 nm 吸光值。以 5% DMSO 取代檢液當空白組，同上述方法測吸光值，所得數值以下方程式計算酪胺酸酶之抑制能力。其中 A 值為反應後空白組吸光值，B 為反應前空白組吸光值，C 值為反應後試驗組吸光值，D 為反應前試驗組吸光值。

$$\% \text{ inhibition} = [(A - B) - (C - D)]\,(A - B)^{-1} \times 100$$

化合物濃度定量分析

化合物濃度定量分析以 UPLC 進行，機型為 Waters ACQUITYTM UPLC system 機型 (Waters Corp., Milford, MA, USA)，分析管柱為 BEH C18 column (reverse-phase chromatographic column, 150 mm × 2.1 mm, 1.7 μm)、檢出器為光電二極體陣列 eλ 偵測器 (Photodiode Array Detector; PDA)，檢測波長 270 nm，管柱溫度 40℃，採梯度流洗 (gradient elution)，在 0 min 時，移動相為酸化水：氰甲烷 = 85 : 15 (v/v)，5 min 時，為酸化水：氰甲烷 = 60 : 40 (v/v)，7 min 時，為酸化水：氰甲烷 = 5 : 95 (v/v)，9 min 時，為酸化水：氰甲烷 = 85 : 15 (v/v)，流速皆為 0.25 mL min^{-1}，樣品注射量 5 μL。酸化水之配製以甲酸滴定去離子水至 pH 3.0，再以 0.2 μm 膜過濾備用。標準品 *trans*-cinnamic acid、ρ-coumaric acid、bergapten 純度 99%，購自 Sigma-Aldrich (Sigma-Aldrich Chemical Co., St Louis, MO, USA)。8-HB 購自 Herboreal Ltd. (Edimburg, UK)，純度 99%。

結果

白芷 *AdC4H* 全長基因序列之選殖與特性分析

為了取得 *AdC4H* 可編譯區之全長序列，由其它植物的 *C4H* 基因設計出一退化性引子 P1 (圖 1)。以 P1 直接對 3’-RACE cDNA 進行

3’-RACE PCR 反應，可選殖出 708 個鹼基對的序列片段，經與其它物種之 *C4H* 比對後，確實具有高相同性。續以此片段設計二引子 P2 及 P3 對 5’-RACE cDNA PCR 增殖反應後，可得 *AdC4H* cDNA 全長序列，含 poly (A) 之 1,760 個鹼基對，可編譯區有 1,518 個鹼基對。

預測可轉譯為 505 個胺基酸，分子量為 57.9 kDa，等電點 pI 為 9.05。*AdC4H* 核酸起始序列周圍呈現 Kozak consensus sequence 特性：在 ATG 上游 −3 為 A、+4 為 G。序列在 5’ 端非轉譯區呈現 A + T rich，確實如文獻所提具植物 Cyt P450 之酵素特質 (Joshi 1987)。以軟體預測發現 AdC4H 之 N- 端有訊息胜肽 (signal peptide)。訊息胜肽之切位點 (signal peptide cleavage sites) 預測有 2 個：第 1 個切位點在第 20 個胺基酸 alanine 和第 21 個胺基酸 isoleucine 之間，第 2 個切位點在第 28 個胺基酸 glycine 和第 29 個胺基酸 lysine 之間 (圖 1)。

胺基酸序列比對結果，AdC4H 和其它的 CYP 相比對有 21%–99% 的相同度，和人

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     ACGCGGGGAACTACACATAAACAAAGCACACACCTAGAACACATCACCTTATAT      54
AAGTAAGTAGTCTCACCCTCAACCAAAACAAACACACACAGAAGAAGAAGAAGAATAATA     114
ATGGATTTTCTGTTGTTGGAGAAGGCCCTGTTGGGCTTGTTCATTGCAACCATTGTAGCC     174
M  D  F  L  L  L  E  K  A  L  L  G  L  F  I  A  T  I  V  A        20
ATAACTATTTCCAAGTTACGTGGCAAGAAACTCAAGCTCCCACCTGGGCCTATTCCCGTT     234
I  T  I  S  K  L  R  G  K  K  L  K  L  P  P  G  P  I  P  V        40
CCTGTGTTCGGTAACTGGTTACAAGTTGGTGATGATCTTAACCAGAGGAACCTGGTGGGC     294
P  V  F  G  N  W  L  Q  V  G  D  D  L  N  Q  R  N  L  V  G        60
TATGCCAAGAAGTTTGGTGACTTGTTTATGTTACGTATGGGACAGAGGAACTTGGTGGTT     354
Y  A  K  K  F  G  D  L  F  M  L  R  M  G  Q  R  N  L  V  V        80
GTGTCTTCACCTGATTTGGCTAAAGATGTTTTGCATACTCAGGGTGTTGAGTTTGGATCA     414
V  S  S  P  D  L  A  K  D  V  L  H  T  Q  G  V  E  F  G  S       100
CGCACTCGTAACGTTGTTTTCGATATCTTTACAGGCAAGGGACAGGACATGGTGTTCACA     474
R  T  R  N  V  V  F  D  I  F  T  G  K  G  Q  D  M  V  F  T       120
GTCTACAGTGAGCATTGGAGGAAGATGAGGAGAATCATGACAGTCCCTTTCTTTACTAAC     534
V  Y  S  E  H  W  R  K  M  R  R  I  M  T  V  P  F  F  T  N       140
AAAGTTGTCCAGCAGTATCGATTTGGGTGGGAGGACGAGGCTGCCCGTGTTGTTGAGGAT     594
K  V  V  Q  Q  Y  R  F  G  W  E  D  E  A  A  R  V  V  E  D       160
GTTAAGGCCAATCCTGAGGCTGCTACCAATGGGATTGTGTTGAGGAACCGATTGCAGTTG     654
V  K  A  N  P  E  A  A  T  N  G  I  V  L  R  N  R  L  Q  L       180
CTCATGTATAATAACATGTACAGAATCATGTTTGATAGAAGGTTTGAGAGCGTAGATGAT     714
L  M  Y  N  N  M  Y  R  I  M  F  D  R  R  F  E  S  V  D  D       200
CCTTTGTTCTTGAAGCTTAAGGCATTGAACGGGGAGCGCAGTAGGCTTGCTCAGAGCTTC     774
P  L  F  L  K  L  K  A  L  N  G  E  R  S  R  L  A  Q  S  F       220
GAGTACAATTACGGAGATTTCATCCCTGTTCTTCGCCCTTTGCTCAAAGGTTATCTCAAA     834
E  Y  N  Y  G  D  F  I  P  V  L  R  P  L  L  K  G  Y  L  K       240
CTCTGCCAGGAAATCAAGGACAAAAGGTTGAAGCTCTTTAAGGATTACTTTGTGGACGAG     894
L  C  Q  E  I  K  D  K  R  L  K  L  F  K  D  Y  F  V  D  E       260
AGGAAGAAGCTTGAAAGCATAAAGAGTGTAGATAACAACGGCTTGAAGTGCGCCATTGAT     954
R  K  K  L  E  S  I  K  S  V  D  N  N  G  L  K  C  A  I  D       280
CACATCTTAGAAGCTCAGCAAAAAGGAGAGATCCACGAGGACAATGTTCTTTACATTGTT    1014
H  I  L  E  A  Q  Q  K  G  E  I  H  E  D  N  V  L  Y  I  V       300
GAAAACATTAATGTTGCTGCAATTGAAACAACACTATGGTCAATTGAATGGGGCATTGCG    1074
E  N  I  N  V  A  A  I  E  T  T  L  W  S  I  E  W  G  I  A       320
GAACTGGTTAATAACCCTGAAATCCAGAAGAAGCTGAGGAATGAGATGGACACTGTGCTT    1134
E  L  V  N  N  P  E  I  Q  K  K  L  R  N  E  M  D  T  V  L       340
GGGGTCGGAGTTCAAATCTGTGAGCCAGACGTCCAGAAGCTCCCTTACCTACAAGCTGTC    1194
G  V  G  V  Q  I  C  E  P  D  V  Q  K  L  P  Y  L  Q  A  V       360
ATCAAAGAGACTCTTCGATATAGAATGGCCATTCCTCTTTTAGTCCCTCACATGAACCTT    1254
I  K  E  T  L  R  Y  R  M  A  I  P  L  L  V  P  H  M  N  L       380
CATGACGCAAAGCTTGCAGGTTACGACATCCCCGCAGAGAGCAAAATCTTGGTCAATGCA    1314
H  D  A  K  L  A  G  Y  D  I  P  A  E  S  K  I  L  V  N  A       400
TGGTGGCTTGCCAACAATCCCGCTCACTGGAAAAACCCAAATGAGTTTAGGCCTGAGAGA    1374
W  W  L  A  N  N  P  A  H  W  K  N  P  N  E  F  R  P  E  R       420
TTCTTGGAAGAAGAATCAAAAGTTGAGGCTAATGGAAATGATTTCAAGTACATACCATTT    1434
E  S  K  V  E  F  L  E  E  A  N  G  N  D  F  K  Y  I  P  F       440
GGGGTTGGGAGGAGAAGCTGCCCTGGAATCATCCTTGCATTGCCTATTCTCGGTATCACA    1494
G  V  G  R  R  S  C  P  G  I  I  L  A  L  P  I  L  G  I  T       460
ATCGGGCGTATGGTGCAGAATTTTGAGCTCTTGCCTCCTCCTGGAATGTCTAAGATTGAT    1554
I  G  R  M  V  Q  N  F  E  L  L  P  P  P  G  M  S  K  I  D       480
ACAGCAGAGAAAGCAGGACAGTTCAGTCTTCAAATTTCGAAGCACTATACTATCGTCTGC    1614
T  A  E  K  A  G  Q  F  S  L  Q  I  S  K  H  Y  T  I  V  C       500
AAGCCAAGATCATTATAAATACTCTGTCTCTTCTACGTTTGTAAAATTGGGTGGACTTGA    1674
K  P  R  S  L  *                                                 505
TACCATTTTGCTACAATATTTTAATAATTTGTGTAAATCTATGAATTTCTAATATGAATG    1734
TTTATTCAAAAAAAAAAAAAAAAAAAA                                     1760
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圖 1. 白芷 *AdC4H* cDNA 與預測之胺基酸序列。核苷酸及胺基酸數目標示於右方，粗體表示核苷酸序列之轉譯起始點及停止密碼，星號表示轉譯終止點，箭頭表示引子，雙底線為 polyadenylation signal。三角形指預測之訊息胜肽切位點。

Fig. 1. Nucleotide and predicted amino acid sequences of *AdC4H* cDNA from *A. dahurica*. The numbers of the nucleotide sequence and amino acid sequence are indicated on the right. The bold letters in the nucleotide sequence indicate start and stop codons. The primers are indicated by arrows. The polyadenylation signal is double underlined. The translation end is marked with an asterisk. The triangle indicates predict cleavage site.

類 Homosapiens 之 CYP3A4 (BAG97065.1) 為最低，只有 21% 相同度 (圖 2)。AdC4H 依 Gotoh (1992) 及 Teutsch *et al*. (1993) 所提可標示 6 個保守性區域 (A–F region) 及 6 個受質辨識區 (圖 2)。Cyt P450 單加氧酶 (monooxygenase) 之重要特性序列，如 N 端具有富含 proline 之 region B (PPGPIPVP)、存在於所有 CYP73A 之酵素中之 Region C (AAIETT) 雙 Ala-Ala 特性 (Ehlting *et al*. 2006) 皆出現於 AdC4H。另一 Cyt P450 重要結構為 region F (PFGVGRRSCPG)，此區的胺基酸 cysteine 供作和 heme 第五配位基結合，因此和其它 CytP450 比對，cyteine 位置相同、完全保留，沒有因親緣遠近或演化而改變為其它胺基酸，此特點在所有生物之 CytP450 皆同 (Werck-Reichhart and Feyereisen 2000)。

AdC4H 融合酵素之功能性表達及特性分析

由於 AdC4H 需要 Cyt P450 reductase 存在才具催化活性，因此，以易取得之阿拉伯芥 cDNA 選殖出 ATR1 完整 cDNA 全長，建

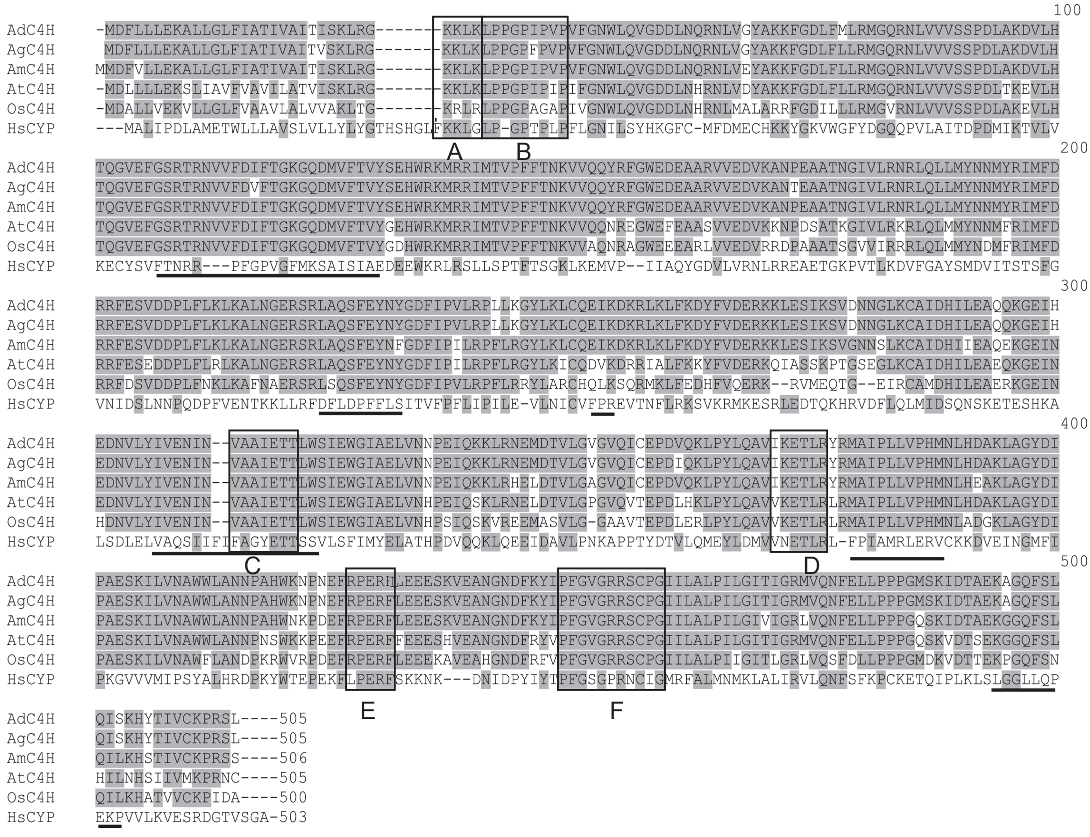

圖 2. 白芷 AdC4H 與其他物種之 C4H 和 CYP 之胺基酸序列比對。序列中以短線填入序列的空隙以得最佳之比對結果。框線為保守性區域，實底線為受質辨識區。其他物種之 C4H 為韓國當歸 (AgC4H, AEA72281.1)、雪珠花 (AmC4H, AAO62904)、阿拉伯芥 (AtC4H, CAP08845.1) 及水稻 (OsC4H, BAG97065.1)。另一為人類 CYP3A4 (HsCYP, BAG97065.1)。

Fig. 2. Amino acid sequences alignment of the AdC4H protein and other C4H and CYP proteins. A dash in the sequence indicates a gap introduced to maintain good alignment. The six highly conserved regions (regions A–F) are indicated by a box. The SRSs are indicated by an underline. The five C4Hs are cinnamate 4-hydroxylase of *A. dahurica* (AdC4H)、*A. gigas* (AgC4H, AEA72281.1)、*Ammi majus* (AmC4H, AAO62904.1)、*Arabidopsis thaliana* (AtC4H, CAP08845.1) and *Oryza sativa* (OsC4H, BAG97065.1). The HsCYP indicated CYP3A4 of Homo sapiens (BAG97065.1).

構 pYES3-ATR1 載體，而 AdC4H 全長可編譯區接合至表現載體 pYES2/NT(B)。二載體 pYES-C4H 及 pYES3-ATR1 共同轉形至酵母菌中，經誘導後，培養於含 *trans*-cinnamic acid 之液體培養基。經 30℃ 培養 20 h 後，可於培養液中檢測得 *trans*-cinnamic acid 及產物 ρ-coumaric acid (圖 3)。經定量分析結果顯示，受質濃度由 100 μM 降低為 38 μM，而產物生成量在三重複試驗後，平均值為 62.9 μM，約為 0.57 mg g^{-1} 細胞重，表示 AdC4H 融合酵素確實在酵母菌中有活性。而對照組 (空載體或只含單一載體之酵母菌) 皆未偵測到產物含量。

為進一步證實酵母菌體中融合 AdC4H 之存在，破菌純化酵母菌體之蛋白質，進行 SDS-PAGE 電泳分離，發現實驗組於 57 kDa 左右有一條帶 (圖 4A)，是控制組所沒有的。接續將膠片轉漬至硝化纖維膜上，以 anti-His-tag 抗體作免疫辨識偵測，可以得到分子量大約為 57 kDa 的蛋白質，和 AdC4H 融合蛋白質預測的大小相近，顯示 AdC4H 確實已成功的在酵母菌表達 (圖 4B)。

為探討 AdC4H 融合酵素之最適 pH，在 25℃ 下以 potassium phosphate 緩衝液涵蓋 pH 4.5–8.5 值作測試，結果顯示 AdC4H 在 pH5.5 即有活性，過了 pH 6.0 活性急速下降，至 pH 8.0 即檢測不到活性 (圖 5)，活性最高之 pH 值位於 pH 6.0。

AdC4H 轉化 bergapten 為 8-HB 之能力與美白能力檢測

為了測試 AdC4H 是否可接受 bergapten 為受質，並將它羥基化為 8-HB (附錄)。經誘導之轉形酵母菌，培養於含 100 μM bergapten 之液體培養基。經 30℃、20 h 培養後，可於培養液中檢測得產物 8-HB，含量為 16.9 ± 2.6 μM，約為 0.44 ± 0.06 mg g^{-1} 細胞重，表示 AdC4H 融合酵素確實可接受 bergapten 為受質並轉化生成 8-HB。培養液經萃取進行美白測試，發現具抗酪胺酸酶活性，IC_{50} 為 1.35 mg mL^{-1}。

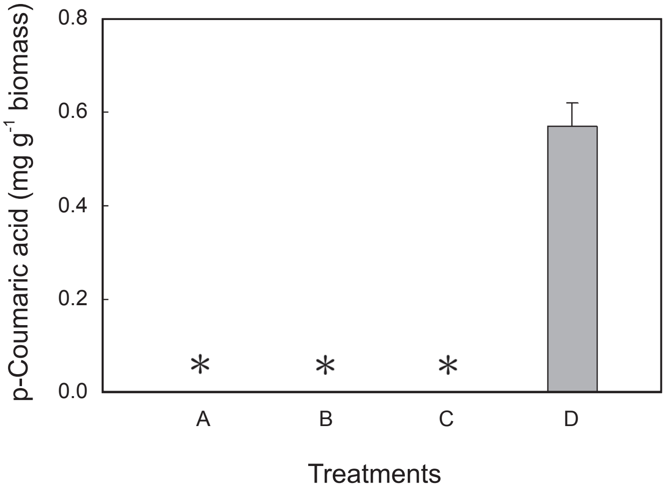

圖 3. 酵母菌轉入不同載體誘導後，以 UPLC 檢測培養液產物 ρ-coumaric acid 之含量。(A) 未接任何基因之 pYES2 空載體；(B) 只轉入 pYES-C4H 一個載體；(C) 只轉入 pYES3-ATR1 一個載體；(D) 轉入 pYES-C4H 及 pYES3-ATR1 二載體。星號表示未檢出。Error bars 指標準差。

Fig. 3. Content of ρ-coumaric acid in the culture of *S. cerevisiae* INVScI containing different vectors. The cells with empty vector pYES2 (A), harboring only pYES-C4H (B), harboring only pYES3-ATR1 (C), or harboring with both pYES-C4H and pYES3-ATR1 (D) were expressed in the presence of substrate. Asterisks mean undetected. The error bars represent standard deviation (SD).

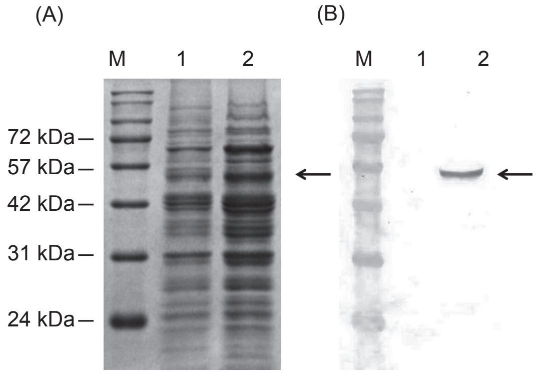

圖 4. 以酵母菌表達 AdC4H 之蛋白質電泳，以 Coomassie blue 染色 (A) 和免疫轉漬圖 (B)。空載體 pYES 之酵母菌 (1) 或接基因 pYES-C4H 之酵母菌 (2)。M：蛋白質標準品，箭頭指 AdC4H 訊號。

Fig. 4. AdC4H was overexpressed in yeast and its separation by electrophoresis (A) and detection by Western blot (B). The yeast cells were harboring empty pYES (1) or pYES-C4H (2) plasmid. M indicates marker proteins. The arrows indicate the overexpressed AdC4H. A signal corresponding to AdC4H was detected with an anti-His tag antibody.

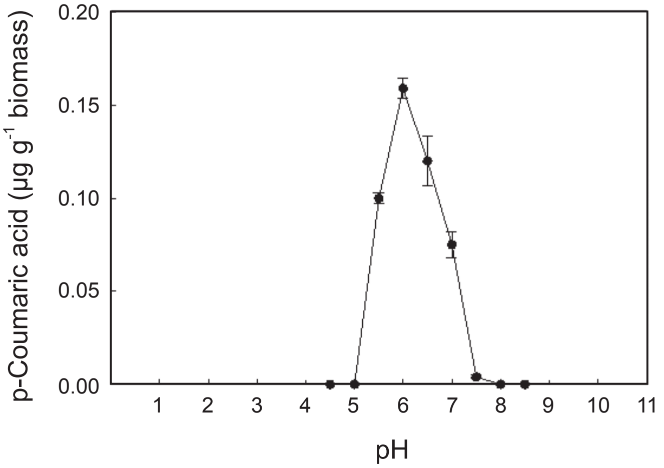

圖 5. 不同酸鹼值之磷酸鉀緩衝液對 AdC4H 融合酵素活性之影響。ρ-coumaric acid 生成量以相同量的酵母菌細胞重為基準比較。每組分析均 3 重複。Error bars 指標準差。

Fig. 5. Effect of different pHs of potassium phosphate buffer on the recombinant AdC4H activity. ρ-coumaric acid is normalized on the basis of an equal amount of yeast cells. Three independent experiments in duplicate were carried out for each analysis. The error bars represent standard deviation (SD).

討論

利用已知它種 *C4H* 序列，本研究成功地取得白芷 *AdC4H* cDNA 全長 1,760 個鹼基對之基因序列。*AdC4H* 胺基酸保守性區域序列具有 Cyt P450 之特性，和其它物種比對下呈現高相同度。在功能性表達顯示需要 CytP 450 reductase，證實屬 Cyt P450 第二群家族之 A-type 一員。

本研究曾嘗試以大腸桿菌為宿主量產融合 AdC4H，但產出之融合 AdC4H 未能偵測得酵素活性，因此依文獻改以酵母菌為宿主，進行融合 AdC4H 之特性研究。由於 C4H 不能自行利用 NADPH 之電子，需要 Cyt P450 reductase 催化 NADPH/NADH 轉移二個電子給 Cyt P450，催化反應方能完成 (Koopmann *et al*. 1999)，因此只含單一載體 pYES-C4H 之

轉形酵母菌，確實未能在含受質之培養基內，檢測得轉化後之產物 ρ-coumaric acid。雖然酵母菌本身就具有 reductase，但對酵素活性試驗分析來說，是不足夠的 (Truan *et al*. 1993；Werck-Reichhart & Feyereisen 2000)。為解決 reductase 不足之問題，本研究採用易取得之 Columbia wild type 阿拉伯芥之 ATR1 reductase 基因，藉由營養篩選培養基，取得具備 pYES-C4H 及 pYES3-ATR1 二載體之轉形酵母菌。誘導後確實成功的檢測得產物 ρ-coumaric acid，含量 62.9 μM，亦證實來自不同物種之酵素，也可協同進行羥基化反應 (Koopmann *et al*. 1999; Osakabe *et al*. 1999; Chen *et al*. 2007)。

本研究確認所建構的轉形酵母菌具 hydroxylase 活性後，續探討此轉形酵母菌是否可將 bergapten 轉化為 8-HB。此構想來自文獻指出，有些 C4H 可催化 *trans*-cinnamic acid 外的其它小分子物質 (如除草劑) (Chen *et al*. 2007)。而白芷的美白成分物質 8-HB，為 bergapten 經 hydroxylase 轉化而來 (附錄)。試驗結果確實在培養液中測得轉化後的目標物，含量 16.9 μM 之 8-HB，美白測試結果亦發現培養液可抑制酪胺酸酶，由此證明融合 AdC4H 可接受 bergapten 為受質。由比對 5 種植物 C4H 胺基酸序列結果發現，所有 C4H 受質辨識區胺基酸組成並非完全一致，序列具多樣性。此多樣性是否和 C4H 可氧化其它分子有所關連，由於酵素結構和其催化活性之關係仍未建立，需進一步探討。發酵所生成的 8-HB 抑制酪胺酸酶能力較學者 Piao *et al*. (2004) 為低，或許可由進一步純化 8-HB 來提高其抑制酪胺酸酶能力。

由 AdC4H 胺基酸序列分析亦可見呈現膜蛋白之特點 (Achnine *et al*. 2004)。首先在 AdC4H 的 N 端，具有疏水性胺基酸佔多數之 membrane anchor 片段，其次為膜蛋白之必要結構-帶正電荷鹼性胺基酸 KKLK 區域 region A (Chapple 1998)。第三為富含 proline (proline-rich) 之 region B (Song *et al*. 1993)。A 與 B 兩區稱為 Hinge，意指形同絞鏈般插在膜上。此些特性因內質網膜之結構為二層帶負電荷之磷脂質所組成，C4H 酵素需靠疏水性胺基酸及帶正電荷之鹼性胺基酸片段插入膜內以牢固結合於膜上，穩定呈現活性以維持正常生理功能運作。另外，文獻指出若將此區域刪除則會使 heme 併入受阻，酵素亦會失去功能 (Koopmann *et al*. 1999)。A 與 B 兩區在動植物之 CytP450 皆存在，可見保留二區域對 C4H 酵素活性之極為重要性 (Yamazaki *et al*. 1993)。

由文獻列舉數種植物之 C4H，如綠豆 (*Phaseolus aureus*)、苜蓿 (*Medicago sativa*)、豌豆 (*Pisum sativum*) 及黃豆 (*Glycine max*)，胺基酸數目介於 503–506 個，分子量亦在 50–60 kDa 之間，和 *AdC4H* 預測可編譯出胺基酸數 505 個及分子量 57.9 kDa 相當。Cyt P450 的 6 個受質辨識區各司其職，SRS1、SRS2 及 SRS4 之 N 端部份胺基酸負責和受質之 aliphatic 區交互作用，而 SRS4 之 C 端部份胺基酸和 SRS5、SRS6 負責與受質之芳香環 (aromatic ring) 接合，而序列中個別胺基酸 N310、I381 及 K494 負責受質之接合和方向定位 (Pina *et al*. 2012)，6 個受質辨識區皆可於 AdC4H 分別標定出，可見不同物種間之 C4H 酵素大小或結構差異性不大 (Kawai *et al*. 1996)。

Cyt P450 依電子傳遞系統之不同分為四群，其中第二群 Cyt P450 之電子傳遞系統需要 Cyt P450 reductase (Rupasinghe *et al*. 2003)。依本研究證實 AdC4H 活性需要 reductase 之結果，將 AdC4H 歸屬於 Cyt P450 第二群家族之一員。另植物 Cyt P450 又分為 A-type 及 non A-type 二種，其中被許多學者用來選殖 A-type 此類基因的三個主要保守區：(V/I) KEx (L/F) R、FxPERF、PFGxGRRxCxG，分別出現在 AdC4H 胺基酸序列之 region D–F，因此將 AdC4H 歸屬於植物 Cyt P450 之 A-type (Bak *et al*. 1998)。依 Ehlting *et al*. (2006) 所提分類，C4H 可依胺基酸的相似度又被細分為被子植物 (angiosperms) 之 Class I 及 Class II (A 和 B)、裸子植物 (gymnosperms) 及苔蘚植物類 (bryophytes)。由本研究結果顯示 AdC4H 可歸於被子植物之 Class I。

C4H 為許多植物二級代謝產物之核心關鍵酵素，代謝衍生出的物質，對人類有重要的經濟價值，藉由了解 AdC4H 基因特性可進一步操控其表現，期未來在工業上可作有效應用。

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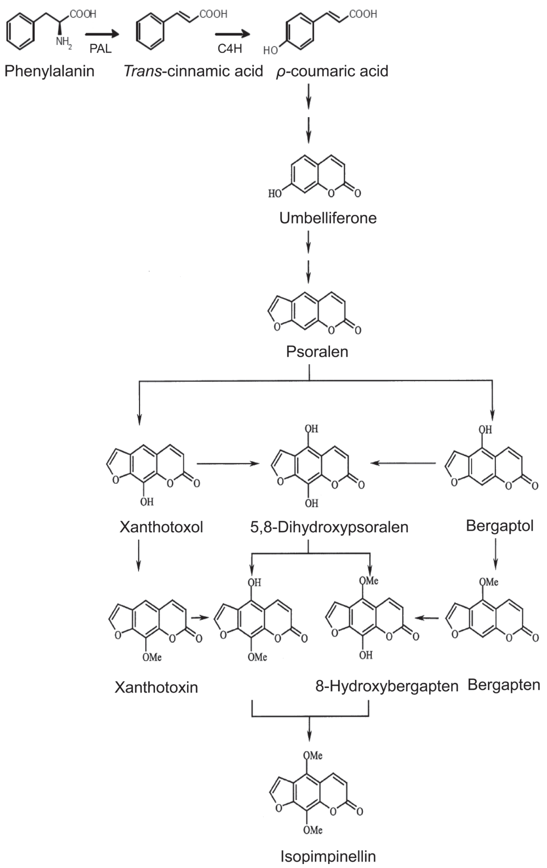
附錄



附錄　呋喃香豆素生合成路徑圖。

Appendix　Supplemental figure. Biosynthesis of linear furanocoumarins (Lo 2013).

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Cloning and Functional Expression of Cinnamate 4-hydroxylase Gene from *Angelica dahurica* (Bai Zhi) and Producing 8-hydroxybergapten in Yesat

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Abstract

Lo, S. C. and P. E. Chung. 2014. Cloning and functional expression of cinnamate 4-hydroxylase gene from *Angelica dahurica* (Bai Zhi) and producing 8-hydroxybergapten in yesat. J. Taiwan Agric. Res. 63(3):205–215.

Cinnamate 4-hydroxylase (C4H) is an enzyme of phenylpropanoids pathway, which can synthesize plant secondary metabolites, including furanocoumarin, a type of pharmaceutical components in Bai Zhi. To characterize *C4H* gene, the complementary DNA of *AdC4H* was cloned from Bai Zhi root using degenerate primers designed from the highly conserved regions of other plant *C4H*. The tandem 5'- and 3'-rapid amplification of cDNA ends via polymerase chain reaction was used to obtain the full-length *AdC4H* cDNA sequences (accession no. KJ453107). The *AdC4H* cDNA contains an open reading frame of 1,518 bp encoding a polypeptide of 505 amino acids with a molecular mass of 57.9 kDa. Amino acid sequence alignment demonstrated over 74% identities on protein level between AdC4H and its homolgous proteins among plant species. They possess all cytochrome P450-featured conserved region A–F and six substrate recognition sites. The full length of *AdC4H* coding region was introduced into pYES2/NT vector. Besides, the full length of cytochrome P450 reductase *ATR1* cloned from *Arabidopsis thaliana* was introduced into pYES3/CT vector. The transformed yeast cell (*Saccharomyces cerevisiae*) harboring two different plasmids (pYES-C4H and pYES3-ATR1) were obtained by growing in the medium without containing tryptophan and uracil. The recombinant AdC4H protein was overexpressed in yeast and partially purified. The recombinant AdC4H revealed optimum activity in pH 6.0 potassium phosphate buffer at 25℃. It suggested that AdC4H together with ATR1 overexpression showed highly efficient catalysis of 4-hydroxylation of cinnamate, the ρ-coumaric acid was increased from undetectable to 62.9 μM. The yeast harboring two plasmids also catalyzed bergapten to 8-hydroxybergapten. The IC_{50} value of tyrosinase inhibition in the medium was 1.35 mg mL^{-1}. The AdC4H enzyme that belongs to a member of cytochrome P450 ClassII family may be a transmembrane protein and its enzyme activity can be detected with reductase from different plant species.

Key words: *Angelica dahurica*, Cinnamate 4-hydroxylase, Cytochrome P450.

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台灣原生花臉香蘑 [*Lepista sordida* (Fr.) Singer] 之馴化

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摘要

李瑋崧、呂昀陞、吳宗諺、蔡淑珍、陳美杏。2014。台灣原生花臉香蘑 [*Lepista sordida* (Fr.) Singer] 之馴化。台灣農業研究 63(3):216–224。

花臉香蘑 [*Lepista sordida* (Fr.) Singer] 為一極珍貴之食藥用菇種，因其栽培的困難度高，現今尚無商業化栽培。本研究自台灣中部地區蒐集 4 個野生花臉香蘑菌株進行馴化培養，經比較溫度和培養基對於供試菌株生長之影響，結果顯示以 24–28℃最適合菌絲之生長。在測試的 5 種培養基中，供試菌株均以在堆肥培養基上的菌絲生長最快，而以馬鈴薯葡萄糖洋菜瓊脂和麥芽酵母粉抽出物洋菜培養基上之菌落紫色較深。進一步利用洋菇稻草堆肥進行栽培，供試菌株皆可順利出菇，其中以菌株 LS-5 出菇最快，從覆土到出菇採收的時間為 22.5 d，其次是 LS-7 需要 25.3 d，LS-11 出菇最慢，需要 73 d。而產量則以 LS-2 最高，平均產量為 2.6 kg 框 $^{-1}$ (每框 15 kg 堆肥)，生物效率 43%，LS-11 的產量最差，生物效率僅有 3.4%。分析 4 個菌株子實體的總多醣、總多酚和總花青素之含量，總多醣含量 LS-2、LS-5 和 LS-7 三菌株約為 13%，明顯高於 LS-11 菌株的 8.76%；然總多酚含量和總花青素含量均以 LS-11 最高，分別為 14.00 mg g $^{-1}$ 乾重和 0.563 mg g $^{-1}$ 乾重。本研究所開發之花臉香蘑量產技術已達商業化水準，且所生產之花臉香蘑具有良好的生理機能成分，未來可進一步將花臉香蘑子實體開發作為保健產品之用。

關鍵詞：花臉香蘑、堆肥、馴化。

前言

花臉香蘑 [*Lepista sordida* (Fr.) Singer] 又名紫晶香蘑、紫晶口蘑、紫花臉蘑、丁香蘑等，隸屬於真菌界 (Fungi)、擔子菌門 (Basidiomycota)、層菌綱 (Hymenomycetes)、傘菌目 (Agaricales)、口蘑科 (Tricholomataceae)、香蘑屬 (*Lepista*) (Hu *et al*. 2006)。花臉香蘑屬於中型菇，白紫至紫色，隨環境或菌齡其紫色可呈現藕紫色或深紫羅蘭色，因其菇傘表面幾乎都有水浸狀紋路，故稱花臉。一般野生者菇傘約 2–10 cm 寬，成熟時菇傘平展，邊緣呈現波浪狀或花瓣狀，中間稍凹；菌褶自菇柄延生，密有小褶，呈淡藍色至淺紅紫色；菇柄長度 2–9 cm、寬度 0.2–1.0 cm，菇柄中生且多呈中空。野生花臉香蘑分布於亞洲、歐洲、澳洲和巴西等地區 (Graf *et al*. 2008)，中國大陸的貴州、黑龍江、遼寧、河北、河南、甘肅、青海、四川、新疆、山西、內蒙古和福建等地亦是主要分布區 (Hu *et al*. 2006)。台灣於晚春至初秋期間在低、中海拔的地區可以發現其蹤跡，包括竹林、草地、灌木叢或富含有機質的田野或庭園間，在日本的高爾夫球場還可見如仙女環般環狀出現 (Terashima & Fujiie 2007)。目前已知全世界香蘑屬菇類超過 150 種，除了花臉香蘑之外，紫丁香蘑 (*Clitocybe nuda* = *Lepista nuda*) 已在歐洲和台灣商業化栽培，其他尚有肉色香蘑 (*Lepista irina*)、灰紫香蘑 (*Lepista*

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glaucicana)、白香蘑 (*Lepista caespitusa*)、粉紫香蘑 (*Lepista personata*) 和濃香蘑 (*Lepista graveolens)* 等均為味道鮮美的食用菌 (Hu & Xie 2008; Li *et al*. 2011)。

花臉香蘑的菇傘肉質柔軟，菇柄有嚼勁，菇體帶有一特殊香氣，近似於薄荷香，味道鮮美。根據 Lu *et al*. (1993) 之分析，野生乾燥的花臉香蘑含有 43% 粗蛋白質、29.37% 碳水化合物、7.58% 粗纖維、2% 粗脂肪，微量元素豐富，菇體生長時可有效富集硒 (Se)、鍺 (Ge)、鋅 (Zn)、鐵 (Fe) 等微量元素，富集硒的能力更是他種菇類作物的幾倍至幾十倍，達 0.93 $\mu g\ g^{-1}$，而鋅的含量可達 84.4 $\mu g\ g^{-1}$ (Luo *et al*. 2003)。除富含麩氨酸 (Glutamic acid) 和天門冬氨酸 (Aspartic acid) 外，更含有 8 種人體必需氨基酸；而菇體所含鎘 (Cd)、汞 (Hg)、鉛 (Pb) 等重金屬含量都低於 1 $mg\ L^{-1}$ (Hu *et al*. 2006; Zhu *et al*. 2011)，符合台灣和美國食品藥物管理局 (FDA) 的規範，惟菇體重金屬所含量除個別菇種吸附能力差異外，尚可隨菇種棲息地或人工栽培基質不同而異，栽培時仍應管控原料所含重金屬量。中國大陸自 1990 年代起，各地即陸續開始對花臉香蘑進行人工栽培研究，但至今仍未能進行量產 (Guo *et al*. 2007; Chen *et al*. 2011b; Liu *et al*. 2012)，原因除菌株特性外，可能還包含栽培本菇之基質與出菇條件尚未能完全掌握，以致此菇在乾菇網拍賣價格為每公斤 200–300 元人民幣不等，屬於極珍貴食藥用菇種。

根據大陸學者報導指出，花臉香蘑具養血、益神、補肝、補五臟等功效 (Hu & Xie 2008; Chen *et al*. 2011a)，此外，花臉香蘑子實體為具保健潛力的抗癌佐劑 (Luo *et al*. 2012)，其水溶性多醣體可經由粒線體路徑 (mitochondrial pathway) 誘發人類咽喉癌細胞 (Hep-2) 產生凋亡 (apoptosis) (Miao *et al*. 2013a; 2013b)；而其菌絲體發酵液被分離出具抗癌能力的 lepistal 和 lepistol 兩種二萜類化合物，其中 lepistal 還能誘導人類白血病細胞分化，並且具有很強的抗細菌和抗真菌能力 (Mazur *et al*. 1996)。花臉香蘑菌絲體發酵液之乙酸乙酯萃取物對於微生物 (包括細菌和真菌) 具良好抑制作用，尤其對於黃瓜萎凋病菌 *(Fusarium oxysporum*) 和辣椒炭疽病菌 (*Colletotrichum capsicibutl*) 之分生孢子抑制效果明顯 (Zhang *et al*. 2010)，對於水稻白葉枯病菌 (*Xanthomonas oryzae* pv. *oryzae*) 亦有顯著的抑制效果 (Liu *et al*. 2012)。

花臉香蘑屬於高價值保健型菇種，除大陸地區已積極進行研發外，日本北海道立綜合研究機構林產試驗場亦開始研究其栽培方法與生理活性 (Personal communication)。本研究利用菇類研究室自台灣各地所蒐集的花臉香蘑菌株，以洋菇稻草堆肥種植，並針對採收後之子實體進行總花青素、總多醣體和總多酚等功能性成分含量分析，期能將之商業化栽培，並進一步作為保健產品開發之材料。

材料與方法

試驗菌株之蒐集與保存

自台灣中部地區蒐集的花臉香蘑代號 LS-2 (台中市霧峰區)、LS-5 (南投縣鹿谷鄉)、LS-7 (台中市霧峰區) 和 LS-11 (台中市霧峰區) 4 個菌株。其中，LS-5 和 LS-7 菌株由彭金騰博士採集，保存於液態氮中，自液態氮中取出後，分別培養於馬鈴薯葡萄糖瓊脂 (potato dextrose agar, PDA; Difco, Maryland USA) 試管斜面上；LS-2 和 LS-11 菌株則自採集的野生花臉香蘑子實體進行組織分離，亦以 PDA 培養基培養。4 個花臉香蘑菌株均於菌絲長滿斜面後保存於 4℃冰箱，定期做繼代培養。

不同溫度對花臉香蘑菌絲生長影響之比較

將上述菌株培養在 PDA 平板培養基上，置於室溫培養 6 d 後，以消毒過的打孔器截取菌落邊緣的菌絲塊 (直徑 8 mm)，之後以消毒過之移植針將其分別移植到 PDA 平板培養基中間(培養皿直徑 9 cm)，每一處理 5 個培養皿，然後分別放置於 4、8、12、16、20、24、28 及 32℃定溫箱中避光培養 1 wk 後，量測菌落直徑，比較不同菌株之菌絲於不同溫度的生長速度。

不同培養基對於花臉香蘑菌絲生長影響之比較

將上述 4 個花臉香蘑菌株培養在 PDA 平板上，以消毒過的打孔器截取邊緣菌絲塊 (直徑 8 mm)，分別移植至堆肥抽出物培養基 (Compost extract agar，CEA，配方為乾堆肥 25 g、碎玉米 100 g、洋菜 20 g、水 1 L)、PDA、麥芽酵母粉抽出物瓊脂 [Malt and yeast extract agar (MYA) 配方為麥芽抽出物 20 g、酵母抽出物 2.5 g、洋菜 20 g、水 1 L] 、麥芽抽出物葡萄糖瓊脂 [Malt extract and glucose agar (MGA) 配方為麥芽抽出物 20 g、葡萄糖 20 g、洋菜 20 g、水 1 L] 和麥芽酵母粉抽出物葡萄糖瓊脂 [Malt extract, yeast extract, and glucose agar (MYGA) 配方為麥芽抽出物20 g、酵母抽出物 2.5 g、葡萄糖 20 g、洋菜 20 g、水 1 L] 等培養基上，每一處理 6 個培養皿，於 25℃定溫箱中培養 1 wk 後，量取菌落直徑。

菌種製作

將麥粒加水煮熟，瀝乾後混合 1% (w/w) 之碳酸鈣，分別秤取100 g裝入四角玻璃瓶中，經高溫高壓 (121℃, 1.2 kg cm^{-2}) 滅菌後備用。將前述長滿 PDA 培養基斜面之菌絲塊以移植針移入滅菌過之麥粒中，於 24℃定溫、避光培養至菌絲長滿瓶內基質為止。

不同花臉香蘑菌株之出菇試驗

洋菇稻草堆肥購自雲林縣周昭陽先生，配方為乾稻草 1,200 kg、青玉米 300 kg、雞糞 100 kg、石膏 60 kg、過磷酸鈣 36 kg、硫酸銨 24 kg 以及碳酸鈣 60 kg，經過室內堆肥發酵方法製成。每框秤取堆肥 15 kg 後，混合 1 瓶上述不同花臉香蘑菌株之麥粒菌種，每一菌株接種 6 框，將接種後的菌框完成編號後以隨機方式放置於環控庫間內，控制室內溫度 24℃、相對溼度 75–80%、無光照和早晚通風 1 次以讓菌絲生長，待 4 wk 後每一框分別覆上約 3 cm 左右厚度的泥炭土 (Mikskaar As, No. Mks1, Estonia)。覆土後室內溫度調整為 18–22℃，二氧化碳濃度降至 1,000 mg L^{-1}，空氣相對濕度設定為 90%，定期補充覆土層表面水份，直到菇體長出至菇傘邊緣反捲前採收秤重。

不同花臉香蘑菌株子實體總多醣體、總多酚和總花青素含量之測定

將上述 4 個花臉香蘑菌株子實體以烘箱烘乾後，以均質機磨成粉末後進行以下之測定，總碳水化合物測定：利用苯酚—濃硫酸法測定 (Dubois *et al*. 1956)，取 0.1 g 乾燥樣品粉末加 5 mL HCl (0.75 N) 於沸水中迴流加熱 2 h，離心取上層液之後，其殘留物再以同樣的方法萃取 1 次，結合 2 次的萃出液，然後以玻璃棉濾紙過濾之後，加水稀釋至 50 mL。然後取萃出液 0.5 mL 加 0.5 mL 苯酚溶液再加 2.5 mL 濃硫酸，作用 30 min 後，測 488 nm 下的吸光值。游離糖含量則以 80% 酒精萃取 2 次，其他同總碳水化合物測定方法，多醣體含量 = (總碳水化合物含量 − 游離糖含量) × 0.9。總多酚化合物測定：取 0.1 g 的乾燥菇體粉末之氫氧化鈉萃取液，加 2.5 mL 水及 0.25 mL Folin-Ciocalteau reagent (2 N) ，最後加入 0.25 mL Na_2CO_3 (20%) 作用 2 h 後，以波長 750 nm 測定吸光值，並以不同濃度之沒食子酸 (gallic acid) 作為對照。總花青素測定則秤取 0.5 g 樣品，加入 10 mL 萃取溶液 (100% 甲醇 + 1% HCl)，混合均勻後於震盪培養箱中以 140×*g*，萃取 30 min 後離心 (4℃，15 min，1,600 g)，取上清液，於 530 nm 測其吸光值 (Huang *et al*. 2006)。

統計分析

以 SAS 統計分析軟體 (SAS® Enterprise Guide 4.1, SAS Institute Inc.) 進行變方分析 (analysis of variance; ANOVA) 後，以最小顯著差異性測驗 (least significant difference test; LSD test) 探討每一試驗各處理間平均值之差異性。

結果

不同溫度對花臉香蘑菌絲生長影響之比較

將花臉香蘑 4 個菌株分別培養在 4–32℃，結果顯示供試菌株之菌絲在 4–32℃下皆可生

長，而最適生長溫度隨菌株而有所差異。菌株 LS-2 以 24℃菌絲生長最快，LS-5 則以 28℃為最適合生長溫度，菌株 LS-7 和 LS-11 在 24 和 28℃下菌絲生長最佳 (圖 1)。

不同培養基對於花臉香蘑菌絲生長影響之比較

比較 5 種不同的培養基對於花臉香蘑菌株菌絲生長之影響，結果發現供試之 4 個菌株都以培養在堆肥抽出物培養基 (CEA) 上生長最快 (表 1)，其次是 MYA 和 PDA 培養基。但如果以菌落呈現紫色的程度而言，培養在 PDA 和 MYA 的菌落顏色最深 (圖 2)，而培養在 CEA 和 MGA 之菌落顏色最淡。以同樣培養在 PDA 培養基上，LS-11 的菌落顏色最深，其次是 LS-2 和 LS-7，LS-5 的顏色最淺。

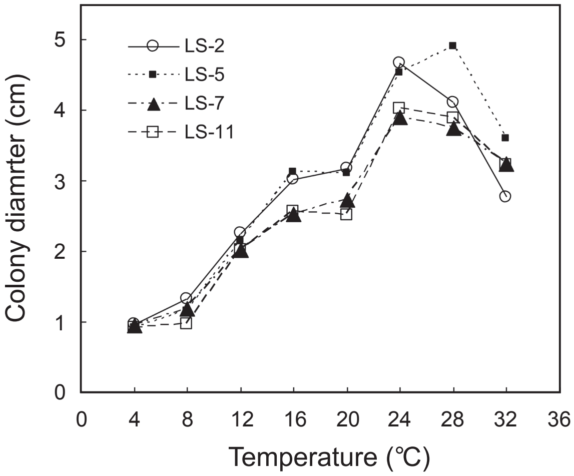

圖 1. 不同溫度對花臉香蘑不同菌株菌絲體生長之影響。

Fig. 1. Effect of temperature on mycelial growth of four strains of *Lepista sordida* grown on PDA after 7 days.

表 1. 不同培養基對不同花臉香蘑不同菌株菌絲生長之影響與花臉香蘑菌絲體在不同培養基生長之紫色程度。

Table 1. Effect of different media on mycelial growth and purple color of colony of four strains of *Lepista sordida* after 7 days incubated at 25℃.

	LS-2		LS-5		LS-7		LS-11	
Medium[z]	Colony diameter (cm)	Color degree[x]	Colony diameter (cm)	Color degree	Colony diameter (cm)	Color degree	Colony diameter (cm)	Color degree
CEA	5.75 ± 0.38 a[y]	-	5.62 ± 0.83 a	-	5.30 ± 0.33 a	-	5.13 ± 0.36 a	-
PDA	4.07 ± 0.10 c	+++	4.42 ± 0.17 bc	++	3.47 ± 0.12 c	+++	3.35 ± 0.10 b	++++
MGA	3.65 ± 0.19 d	-	3.50 ± 0.15 d	-	2.85 ± 0.08 d	-	2.35 ± 0.16 d	-
MYA	4.83 ± 0.23 b	++++	4.78 ± 0.17 b	++	4.48 ± 0.15 b	++	3.50 ± 0.32 b	+++
MYGA	4.08 ± 0.13 c	++	4.13 ± 0.05 c	+	3.47 ± 0.08 c	+	2.95 ± 0.10 c	++

[z] CEA = compost extract agar; PDA = potato dextrose agar; MGA = malt extract and glucose agar; MYA = malt and yeast agar; MYGA = malt extract, yeast extract, and glucose agar.

[y] Values are mean ± standard error of mean, n = 6. Means with the same letters of a column are not significantly different at 5% level by least significant difference (LSD) test.

[x] The symbol “ - ” means no purple color. The symbols “ + ”, “ ++ ”, “ +++ ”, and “ ++++ ” mean the density of purple color (light to dense).

不同花臉香蘑菌株之出菇試驗

在花臉香蘑之栽培試驗中，供試之 4 個菌株在菌絲走菌的時間上差異不大，但出菇所需時間和產量則因菌株而有所不同。其中，LS-5 菌株最早出菇 (圖 3)，從覆土到第一次採收所需時間為 22.5 d (表 2)，其次是 LS-7 菌株 25.3 d 和 LS-2 菌株 28.3 d，而以 LS-11 菌株出菇最慢，需要 73 d。統計出菇情形，LS-2 菌株產量最高，平均每框 (15 kg 堆肥) 可達 2.6 kg (表 2)，生物效率可達 43.5%，LS-11 菌株產量最差，平均每框產量只有 206 g，生物效率只有 3.4%。

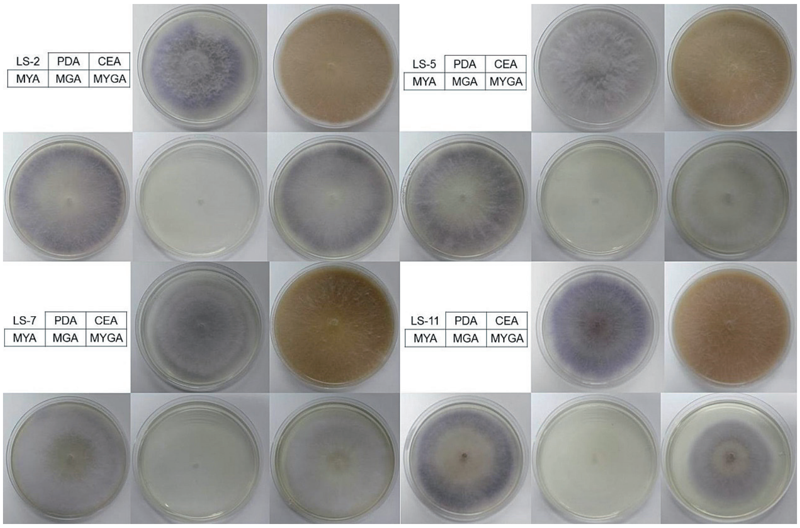

圖 2. 不同花臉香蘑菌株之菌絲體培養於不同培養基之形態。

Fig. 2. The mycelia of four *Lepista sordida* strains cultured on different media at 25℃ after 7 days.

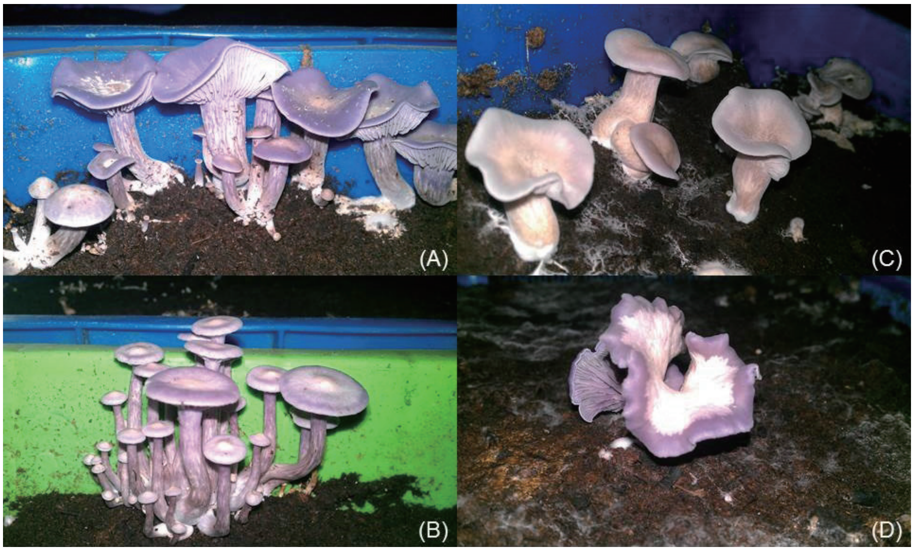

圖 3. 四個花臉香蘑菌株栽培於堆肥之子實體形態。

Fig. 3. Fruiting bodies of four *Lepista sordida* strains, LS-2 (A), LS-5 (B), LS-7 (C), and LS-11 (D), grown on indoor composts.

表 2. 不同菌株之花臉香蘑之產量及生物效率比較。

Table 2. Comparisons of effects of compost on yield and biological efficiency of different strains of *Lepista sordida*[z].

Strain	Yields of each flush (g) pot^{-1}				Total yield (g) pot^{-1}	Biological efficiency (%)[y]	Days from casing to 1st harvest
	1st flush	2nd flush	3rd flush	4th flush			
LS-2	822.3	906.5	817.3	61.8	2607.9 ± 287.7 a[x]	43.5 ± 4.8 a	28.3 b
LS-5	399.0	48.5	296.3	0	743.8 ± 551.9 b	12.4 ± 9.2 b	22.5 c
LS-7	580.1	0	548.8	11.8	1140.7 ± 259.3 b	19.0 ± 4.3 b	25.3 b
LS-11	0	101.0	105.3	0	206.3 ± 162.7 c	3.4 ± 2.7 c	73.0 a

[z] The compost was composed of 1,200 kg of dry straw, 300 kg of green corn, 100 kg of chicken manure, 60 kg of calcium carbonate, 60 kg of gypsum, 24 kg of ammonium sulfate, and 36 kg of calcium superphosphate before composting. Each pot contained 15 kg compost of which water content and pH were 73% and 6.8, respectively. There were 6 replicates for each strain.

[y] Biological efficiency (%) = (the weight of fresh mushroom harvested/dry matter content of substrate before inoculation) × 100.

[x] Values are mean ± standard error of mean, n = 6. Means with the same letter of a column are not significantly different at 5% level by least significant difference (LSD) test.

不同菌株子實體總多醣體、總多酚和總花青素含量測定

比較 4 個花臉香蘑菌株子實體的花青素含量，結果以 LS-11 的花青素含量最高，平均為 0.563 mg g^{-1} (乾重)，其次是 LS-2 (表 3)，LS-5 和 LS-7 的花青素含量最低。總多醣的含量部份，以 LS-11 含量最低，平均為 8.76% (乾重)，而 LS-7 含量最高，其次依序為 LS-5 和 LS-2，但三者無顯著差異。總多酚的含量測定則以 LS-11 的 14.00 mg g^{-1} (乾重) 最高，其次依序為 LS-7、LS-5 及 LS-2。

討論

Terashima & Fujiie (2007) 研究指出日本花臉香蘑菌株的生長溫度範圍為 10–30℃，最適生長溫度為 25℃，Hu & Xie (2008) 也發現大陸花臉香蘑菌株 Le.s0529-1 菌絲生長溫度為 8–32℃，最適生長溫度為 22–25℃，低於 5℃菌絲不生長；本研究測試 4 個花臉香蘑菌株，發現菌絲在 4–32℃均可生長，最適生長溫度隨著菌株的不同而有差異，但仍以 24–28℃為最適合生長溫度，此結果與前人研究接近。然所分離出之花臉香蘑菌株可耐低溫至 4℃，由於台灣地處亞熱帶，環境溫度較中國大陸和日本多數地區為高，部分菌株於 28℃生長良好，可能是菌株開始產生適應性的分化所致。

在測試的 5 種培養基中，供試菌株均以培養在堆肥抽出物培養基 (CEA) 上的菌絲生長最為快速，此特性與其野生發現之環境相符 (其野生多發生於腐植土之環境相符)。Tian *et al*. (2003) 比較生料和熟料對於花臉香蘑出菇之影響，認為熟料覆土袋栽法是最佳生產模式。Hu & Xie (2008) 亦發現花臉香蘑之菌絲在經發酵處裡的培養基質中，其生長速度明顯優於未發酵的材料。由於花臉香蘑普遍出現在富含腐植質的田野、菜園或坡地上，顯示該菌偏好腐熟過後的營養，因此學者多建議將基質進行發酵

表 3. 花臉香蘑不同菌株子實體總多醣體、總多酚及總花青素含量之比較。

Table 3. Comparison of total polysaccharides, polyphenols, and anthocyanins in fruiting bodies of different strains of *Lepista sordida*.

Strain	Total polysaccharides[z] (%)	Total phenols (mg g^{-1})	Total anthocyanins (mg g^{-1})
LS-2	13.41 ± 1.28 a[y]	10.66 ± 0.33 d	0.487 ± 0.006 b
LS-5	13.57 ± 0.60 a	11.23 ± 0.25 c	0.453 ± 0.011 c
LS-7	13.81 ± 0.59 a	12.71 ± 0.27 b	0.453 ± 0.011 c
LS-11	8.76 ± 1.20 b	14.00 ± 0.29 a	0.563 ± 0.012 a

[z] Dry weight of fruiting bodies.

[y] Values are mean ± standard error of mean, n = 3. Means with the same letter of a column are not significantly different at 5% level by least significant difference (LSD) test.

後方作為花臉香蘑栽培介質。本研究以室內發酵方式生產之稻草堆肥進行出菇試驗，結果供試菌株中以 LS-2 生物效率最高，可達 40% 以上， 是 Tian *et al*. (2003) 的 2.19 倍、Hua *et al*. (2008) 的 2.18 倍；而本研究使用之栽培框的裝料面積為 0.2204 m^2 (58 cm × 38 cm)，將其平均產量為每框 2,607.9 g，換算後得每平方公尺產量約為 11.83 kg m^{-2}，約是 Tian *et al*. (2003) 產量試驗結果的 3 倍、Guo *et al*. (2007) 產量試驗結果的 5.4 倍；整體而言，LS-2 菌株的出菇情形已達商業化生產的水準，而本研究亦顯示以目前之室內堆肥發酵技術所生產之堆肥，確實可用於生產花臉香蘑之用。

花臉香蘑和紫丁香蘑兩者均是紫色菇類，「台農一號」紫丁香蘑為行政院農業委員會農業試驗所自法國引進母種後自行育成，已獲得專利並技轉給農民種植。紫丁香蘑屬於低溫型菇類，出菇最適溫度為 13–15℃，而花臉香蘑屬於中溫型菇類，子實體最適合發育的溫度是 20–26℃，溫度高於 32℃或低於 15℃時，不易產生菇蕾 (Tian *et al*. 2003)。比較兩者的產量，紫丁香蘑最高產量平均每框 15 kg 堆肥可達 2.6 kg (Chen *et al*. 2003)，和本試驗中 LS-2 菌株的產量相近，顯示栽培花臉香蘑取代紫丁香蘑，將可省下部份電費成本。Tian *et al*. (2003) 建議花臉香蘑的播種量應高於培養料乾重的 15% (Tian *et al*. 2003)，本研究發現每框 15 kg 的堆肥只需約 100 g 的菌種，占栽培基質鮮重 0.6%，遠低於紫丁香蘑菌種量須達 3%。換算成栽培基質乾重比約在 2.5–3.0%，下種量和洋菇接近，顯示在投入菌種之成本上花臉香蘑也較為低廉。

近幾年菇類的機能性成分獲得高度重視，被視為開發新藥品的重要來源。紫丁香蘑熱水萃取物經動物試驗證實可以活化免疫系統 (Chen *et al*. 2013)，同時具有降血脂和血糖之功效 (Chen *et al*. 2014)。紫丁香蘑菇體的多醣體含量為 12.62%，總花青素的含量為 0.045%，總多酚化合物含量為 1.29% (Chen *et al*. 2014)。本研究分析花臉香蘑的多醣體含量為 13.81%，總多酚含量可高達 1.4%，總花青素含量為 0.056%，兩種菇類所含花青素、多醣和多酚含量相近。此些結果顯示，花臉香蘑具有相當良好之功效性成分，為具潛力可開發作為保健產品之菇類。至於 LS-11 菌株的總多酚和總花青素含量較高，值得進一步改良栽培技術，提高單位面積產量。

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Domestication of Native *Lepista sordida* (Fr.) Singer in Taiwan

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Abstract

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Lepista sordida (Fr.) Singer is an important edible and medicinal mushroom. Due to its difficulty of cultivation, it has not been produced commercially. Four strains of *L. sordida* collected from central Taiwan were evaluated for their optimum mycelial growth temperature, and growth media, yield, biological efficiency and bioactive compounds of fruiting bodies. In relation to the growth temperature, results showed that 24–28℃ was the most suitable temperature range for mycelial growth of these four strains of *L. sordida*. Among the five media tested, mycelia of all the four strains grew fastest on compost extract medium, but the purple color of colony was darker on potato dextrose agar (PDA) and malt and yeast agar (MYA). Furthermore, these four stains were spawned in indoor compost and produced fruiting bodies successfully. Strain LS-5 fruited earliest, taking 22.5 d from casing to the 1st harvest, followed by LS-7 and LS-11 strains, taking 25.3 d and 73.0 d, respectively. Strain LS-2 was the most productive one with the highest yield of 2.6 kg pot^{-1} (with 15 kg compost) and a biological efficiency of 43%, while the yield of the strain LS-11 was the lowest with a biological efficiency of only 3.4%. In order to compare the bioactive compounds existed in the fruiting bodies of the four strains, the total contents of polysaccharides, phenols and anthocyanins were analyzed. Results showed that the contents of total polysaccharides in the strain LS-2, LS-5 and LS-7 were significantly higher than that of the strain LS-11. However, the total content of both phenols and anthocyanins in the strain LS-11 were higher than those of the other three strains (14.00 mg g^{-1} dry weight and 0.56 mg g^{-1} dry weight, respectively). Because the yield of the strain LS-2 has reached the level of commercial production and it contains a relative amount of bioactive compounds in its fruiting bodies, this mushroom strain would have the potential to be cultivated on a commercial scale and used as the source for the exploitation of functional supplements in the near future.

Key words: *Lepista sordida*, Compost, Domestication.

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超甜玉米鮮果穗產量及其它農藝性狀之遺傳分析

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摘要

劉紹國、謝光照。2014。超甜玉米鮮果穗產量及其它農藝性狀之遺傳分析。台灣農業研究 63(3):225–234。

以 9 個商業品種分離至 S_5 之自交系及半互交之 36 個 F_1 組合為材料，進行鮮果穗產量及其它農藝性狀之遺傳分析。親本自交系中 4 個屬熱帶型及 5 個為溫帶型品種。調查之性狀包括開花期、吐絲期、株高、稈徑、總葉數、葉面積、含苞葉果穗重、去苞葉果穗重、穗長、穗徑等。試驗之數據經以 Hayman (1954a, 1954b) 之全互交分析法進行性狀遺傳介量與基因作用效應分析結果：植株營養性狀共同受累加性及顯性所控制，顯性效果大於累加性效果。基因作用之平均顯性程度以超顯性居多，控制各性狀之顯性基因群數有 4–6 群，其顯性基因都屬增量基因。開花與吐絲期性狀共同受累加性及顯性所控制，而其累加性效果大於顯性效果；顯性作用大部分係正負效應基因頻度分布不均所造成，控制開花與吐絲期之顯性基因群數有 3–5 群，其顯性基因為減量基因，且屬不完全顯性。而含苞葉鮮穗重、穗長與去苞葉鮮穗重、穗徑之性狀共同受累加性及顯性所控制，顯性效果大於累加性效果，平均顯性程度為超顯性，控制之顯性基因群數分別有 5–6 與 3–4 群，其顯性基因均為增量基因。

關鍵詞：超甜玉米、全互交、遺傳介量、基因作用效應。

前言

超甜玉米鮮穗果產量與品質是育種之重要項目，而鮮穗產量是受多基因影響的數量性狀，其它農藝性狀與產量關係也是國內外育種者關心的重要課題。Zeng *et al*. (2008) 以 6 個超甜玉米自交系組合，探討花期性狀的遺傳，指出超甜玉米花期性狀的遺傳符合加性 - 顯性模式，超甜玉米花期性狀的遺傳均方由加性效應和顯性效應共同組成，顯性效應比加性效應更為重要，而且存在超顯性現象，控制花期性狀的基因至少有 3 對以上。Cal *et al*. (2002) 認為穗位葉葉面積符合加性-上位性遺傳模型，Wun *et al*. (2002) 認為穗位葉葉面積加性變方大於顯性變方，一般組合力表現出比特殊配合力較大的遺傳份量。Zhou *et al*. (2008) 探討不同生長時期之葉面積，指出玉米葉面積主要受到顯性遺傳效應的影響。上述結果皆說明穗位葉葉面積的遺傳基本上屬於數量遺傳。

Mariani & Desiderio (1975) 利用義大利硬粒種族群所分離自交系，行全互交組合分析發現，產量及相關性狀受累加性及顯性基因共同作用所控制。而馬齒型與硬粒型種源間之分析結果顯示顯性基因的作用大於累加性基因之作用 (Moreno-Gonzalez & Dudley 1981)。而 Lee *et al*. (1986) 指出一般硬粒種自交系具有較佳之一般組合力，而馬齒種自交系則具有較佳的特殊組合力。Malvar *et al*. (1996) 也指出產量主要受顯性變方所影響。Wang *et al*. (2005) 以 6 個鮮穗產量表現差異顯著的超甜玉米自交系為材料，研究指出超甜玉米鮮穗產量遺傳具有多樣性，而且存在非等位基因間的作用方向為正效應 , 即對鮮穗產量有增加效

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應，是顯性基因起主導作用，分佈位點上不對稱，即在參試親本中，正負基因分佈是不均勻的，同時親本帶有的顯性基因頻率大於隱性基因頻率，而控制鮮穗產量的顯性基因對數至少有 4 對，控制鮮穗產量的狹義遺傳力僅為 17.83%。而 Wu *et al*. (2006) 以黑甜玉米為材料，顯示鮮果穗重及穗徑主要係受累加性基因控制，穗長與粒數受加性及顯性基因共同控制。Liu *et al*. (2008) 以超甜玉米為材料研究結果顯示，果穗性狀受到了加性基因和非加性基因的控制。其中鮮穗重、穗行數、穗徑的一般配合力均方大於特殊配合力均方，表示這些性狀在雜交組合中的遺傳效應中以加性基因效應為主。穗長和行粒數的特殊配合力均方大於一般配合力均方，說明這兩個性狀在雜交組合中的遺傳效應中以顯性效應為主。並指出同一性狀不同材料間，同一材料不同性狀間其配合力效應表現複雜，存在不同效應大小和正負作用方向的差異。Shieh & Thseng (2002) 以栽培在台灣不同地區的台南白玉米族群自交分離之自交系及其相互雜交而成之半互交 F_1 組合為研究材料，以 Hayman (1954a, 1954b) 之方法分析結果顯示開花期、吐絲期、株高、穗位高、果穗乾重、籽粒乾重、穗長、穗徑等性狀皆呈現受累加性基因控制外，也受複雜的顯性基因控制，除穗長之外，其他性狀均呈現顯性效果大於累加性效果，並有 5 至 8 個顯性基因群控制其顯性效果之表現。其中穗長、穗徑、果穗乾重及籽粒乾重受 6 群基因控制，且顯性基因具增量效果。對玉米產量及其它農藝性狀在基因效應與遺傳介量分析的結果，由於材料的不同而產生不同的結果。而國內甜玉米相關遺傳研究較缺乏，文獻也較少。針對甜玉米產量及其它農藝性狀之遺傳分析，實有加以探討的必要性。

材料與方法

本研究以 9 個商業品種分離至 S_5 之自交系及半互交之 36 個 F_1 組合為材料，進行鮮果穗產量及其它農藝性狀之遺傳分析。其中 4 個為熱帶型自交系 (PH5-5、BJ12、PH10-23、SH-1)，5 個為溫帶型自交系 (GH-8、Venus 8、Honey 236-1、HJ 2-7、SN 3-8) (表 1)。田間試驗於 2006 年 1 月於南投集集鎮農家田區進行。田間設計為逢機完全區集設計 (RCBD)，3 重覆，行長 4.5 m，3 行區，行株距為 80 cm × 30 cm。公頃三要素肥料量為 $N-P_2O_5-K_2O$ = 180：90：60，基肥以台肥 39 號複合肥料 ($N-P_2O_5-K_2O$ = 12：18：12) 每公頃施 500 kg，即基肥每公頃施氮素 60 kg，磷肥 90 kg 及鉀肥 60 kg。其餘之氮素 120 kg 以硫酸銨 (N = 21%) 於玉米長至齊膝期 (約 50–60 cm) 當追肥施用。其餘之田管理按實際需要進行之。

生育期間調查開花期、吐絲期、株高、稈徑、總葉數、葉面積、含苞葉果穗重、去苞葉果穗重、穗長、穗徑等。調查方法如下：開花期 (days to tasseling)：指播種日起至小區中 50% 植株達雄穗始花所需日數。吐絲期 (days

表 1. 試驗材料一覽表。

Table 1. Inbred lines of super sweet maize used in the study.

Inbred line	Type	Introduce company of original variety
PH 5-5	Tropical	TAIPEI NONG CI CO.
BJ 12	Tropical	KNOWN-YOU SEED CO., LTD.
PH10-23	Tropical	TAIPEI NONG CI CO.
SH-1	Tropical	Taiwan Agricultural Research Institute (TARI)
GH-8	Temperate	BO YOU CO., LTD
Venus-8	Temperate	BO YOU CO., LTD
Honey 236-1	Temperate	SINON CORPORATION
HJ2-7	Temperate	KNOWN-YOU SEED CO., LTD.
SN 3-8	Temperate	SINON CORPORATION

to silking)：指播種日起至小區中 50% 植株達雌穗開始吐絲所需日數。株高 (plant height)：於植株雄穗開花後，每小區逢機 5 株，由地面至雄穗頸節之平均高度，以 cm 表示。稈徑 (stalk diameter)：於植株雌穗吐絲後，每小區逢機 5 株，量取地面第一節中央的平均直徑，以 mm 表示。總葉數 (total leaf number)：於植株雄穗抽出後，選 5 株調查單株之總葉數，以 No. $plant^{-1}$ 表示。葉面積 (leaf area per plant)：由頂端往下數第 8 片葉的長度與最寬的乘積，再乘以 0.75 × 9.39 所獲得 cm^2 數值 (Pearce *et al*. 1975)。含苞葉鮮果穗重 (ear fresh weight with husk)：逢機選取乳熟期之 5 個果穗，稱其含苞葉果穗鮮重，以 g ear^{-1} 表示。鮮果穗重 (ear fresh weight)：上述乳熟期逢機之 5 個去苞葉後稱其果穗鮮重，以 g ear^{-1} 表示。穗長 (ear length)：上述乳熟期之 5 個逢機果穗，量其整穗長度之平均值，以 cm 表示。穗徑 (ear diameter)：上述乳熟期之 5 個逢機果穗，量其果穗中間部位之平均值以 mm 表示。

所獲數據以 Hayman (1954a, 1954b) 方法進行遺傳介量估算及基因作用效用分析。各參數所代表的意義為：(1) a 累加性作用 (親本間的遺傳變異)。(2) b 顯性效果 (各雜交組合間的之顯性效果)。(3) b_1 為平均顯性效果 (平均顯性效果對 b 顯性效果的影響)。(4) b_2 為顯性效果是否由親本所帶有之遺傳因子頻度不相等所造成。(5) b_3 為顯性效果是否為 SCA 所造成 (特殊組合力對顯性效果的影響)。(6) D 為基因之累加性作用。(7) H_1 為基因之顯性作用。(8) H_2 為組合的顯性作用。(9) h^2 值為所有雜交組合之顯性效果。(10) D-H_1，> 0，累加性效果大於顯性效果；< 0，顯性效果大於累加性效果。(11) E 為環境作用。(12) $(H_1/D)^{1/2}$ 為平均顯性程度：> 1.3 (超顯性)、0.76–1.30 (顯性)、0.26–0.75 (不完全顯性)、< 0.25 (無顯性)。(13) H_2/4 H_1 為親本正負效應之比值，偏離 0.25 表顯性作用大部分是正負效應基因頻度分布不均所造成 。(14) Kd/Kr 為顯隱性基因之比值，= 1，表示顯隱性基因數目約同；< 1，隱性基因數目多；> 1，顯性基因數目多。(15) h^2/H_2 為控制各性狀之顯性基因群數。(16) 親本間 Wr + Vr 值為親本間的顯性順序。(17) Yr，親本外表型觀測值。(18) Yr 與 (Wr + Vr) 之相關係數 (r)：r 為顯著負值，表示顯性基因為增量基因；r 顯著正值，表示顯性基因為減量基因；r 不顯著，表示顯性基因作用無一定方向。(19) Wr/Vr 之迴歸及拋物線：Wr/Vr 之迴歸線交於 Wr 軸原點以上，表示基因之顯性程度為不完全顯性作用；交於 Wr 軸原點上，表示基因之顯性程度為完全顯性作用；交於 Wr 軸原點以下，表示基因之顯性程度為超顯性作用。

結果

以 9 個品種之自交系及其半互交之 F_1 雜交種為材料，進行 10 個農藝性狀調查，經刪除非均質之親本後 (具有互補基因)，利用 Hayman (1954a, 1954b) 之方法進行變方分析結果，全部性狀在組合間皆達極顯著差異。在累加性作用 (a) 上，全部性狀皆呈極顯著，表示各性狀親本間存在極大的遺傳變異。在顯性效果 (b) 上，全部性狀皆呈極顯著，表示各性狀其雜交組合存在顯性效果。於平均顯性效果 (b_1) 上，開花期、吐絲期、株高、稈徑、總葉數、葉面積、含苞葉果穗重、穗長、穗徑等達顯著，其餘性狀則皆達極顯著，表示平均顯性效果對顯性效果有明顯的影響。b_2 變因，在總葉數、去苞葉果穗重、穗徑不顯著；而開花期、吐絲期、株高、稈徑、葉面積、含苞葉果穗重、穗長皆達極顯著，表示除總葉數、去苞葉果穗重、穗徑外，其他性狀顯性效果受到親本所帶有之遺傳因子頻度不相等所造成。b_3 變因，在總葉數與穗徑達顯著；開花期、吐絲期、株高、稈徑、葉面積、含苞葉果穗重、去苞葉果穗重、穗長達極顯著，表示各性狀之顯性效果受到特殊組合力 (SCA) 的影響 (表 2)。

分析遺傳介量結果，顯示在 D 變因，除穗徑未顯著外，其餘皆達極顯著，表示除穗徑外，其餘性狀受基因之累加性作用控制性狀之表 現 (additive effect of gene)。H_1、H_2 及 h^2 變因在各性狀皆極顯著，表示基因之顯性作用、組合的顯性作用及所有雜交組合之顯性效果都有顯著影響。而 D-H_1 變因，除開花期、吐絲期 > 0，表示其累加性效果大於顯性效果

表 2. 超甜玉米鮮果穗產量及其他農藝性狀全互交變方分析之均方值。

Table 2. Mean squares from the analysis of variance for plant and ear characters of the diallel cross set.

Variation	Days to tasseling	Days to silking	Plant height	Stalk diameter	Total leaf number	Leaf area per plant	Ear fresh weight with husk	Ear fresh weight	Ear length	Ear diameter
Block	200**	155**	55	21**	1.7	18	20	23	0.5	10.4
Genotype	114**	113**	426**	22**	4.3**	951**	263**	126**	21.8**	44.9**
a	729**	759**	1,545**	124**	22.5**	6,059**	1,089**	96**	82.5**	34.2**
b	91**	83**	572**	21**	4.6**	768**	343**	265**	30.1**	93.5**
b_1	1,504*	1,560*	11,336**	368*	80.2**	19,165*	9,118*	2,522**	819.7*	1,233.3*
b_2	68**	74**	221**	21**	3.2	304**	100**	7	12.6**	11.6
b_3	46**	31**	157**	8**	2.2*	224**	90**	20**	6.0**	12.4*
Error	9	10	12	2	1.1	35	9	4	0.5	3.5
Code be cancelled	---	---	3	----	----	----	---	2, 4, 5, 8	----	1, 2, 3

*,**Significant at 5% and 1% probability levels, respectively.

外；其餘性狀皆 < 0，顯示其它性狀之顯性效果大於累加性效果。另受環境作用 (E) 上，在開花期、吐絲期及稈徑達顯著以上之差異，表示這 3 個性狀容易受環境影響；其餘株高、總葉數、葉面積、含苞葉果穗重、去苞葉果穗重、穗長及穗徑等不易受環境影響。各性狀之 $(H_1/D)^{1/2}$ 值 (平均顯性程度)，其中 > 1.3 者有株高、總葉數、葉面積、含苞葉果穗重、去苞葉果穗重、穗長及穗徑，表示基因作用程度為超顯性；介於 0.76–1.3 者有開花期、吐絲期及稈徑，表示基因作用程度為顯性。$H_2/4\ H_1$ 值 (親本正負效應之比值)，除去苞葉果穗重 (0.249) 未偏離 0.25 外，其餘都偏離 0.25，顯示除去苞葉果穗重外，其餘性狀之顯性作用大部分是正負效應基因頻度分布不均造成的，即指顯隱性基因在親本的基因座上分布頻度不均勻對顯性作用產生明顯的作用。Kd/Kr 為顯隱性基因之比值，穗徑 = 1，表示顯隱性基因數目約同；株高、葉面積、含苞葉果穗重及去苞葉果穗重等皆 < 1，表示其隱性基因數目多；開花期、吐絲期、稈徑、總葉數及穗長等皆 > 1，表示其顯性基因數目多。h^2/H_2 值為控制各性狀之顯性基因群數，其中開花期、稈徑、去苞葉果穗重、穗徑為 5–6 及行數有 3–4 群基因控制性狀的表現；吐絲期、株高及總葉數有 4–5 群基因控制其性狀的表現；穗位高、葉面積、含苞葉果穗重及穗長有 5–6 群基因控制其性狀的表現。而各性狀之狹義遺傳率分別為開花期與吐絲期約在 60% 上下，葉面積與稈徑之狹義遺傳率約為 50%，株高、總葉數及含苞葉果穗重狹義遺傳率約為 40% 上下，去苞葉果穗重與穗徑之狹義遺傳率約為 10% 上下。親本間的顯性順序 (Wr + V) 與親本外表型觀測值 (Yr) 之相關係數 r，其中 r 為顯著負值者有株高、稈徑、總葉數、葉面積、含苞葉果穗重、去苞葉果穗重、穗長及穗徑，表示其顯性基因為增量基因。而開花期、吐絲期之 r 值為顯著正值，表示其顯性基因為減量基因 (表 3)。

開花期、吐絲期與總葉數等性狀之 Wr/Vr 迴歸及拋物線結果 (圖 1)，顯示其所有的點都在拋物線內，迴歸直線交於 Wr 軸原點以上，為不完全顯性作用。稈徑性狀之 Wr/Vr 迴歸及拋物線結果，顯示其所有的點都在拋物線內，迴歸直線交於 Wr 軸原點，為完全顯性作用。株高、葉面積、含苞葉果穗重、去苞葉果穗重、穗長及穗徑等性狀之 Wr/Vr 迴歸及拋物線結果，顯示其所有的點都在拋物線內，迴歸直線交於 Wr 軸以下，為超顯性作用。

依 Hayman (1954a, 1954b) 的全互交理論，親本之標準化偏差值與正同質體 (positive homozygote) 之數目有密切關係，且顯性次序 (Wr + Vr) 也與隱性同質體 (recessive homozygote) 之數目相關。所以利用標準偏差法估算顯性次序與親本計量 (Yr) 的相對位置。開花

表 3. 超甜玉米農藝性狀全互交分析之遺傳變方成分估值。

Table 3. Estimated value of the genetic variance components in the diallel crosses set.

Genetical component	Days to tasseling	Days to silking	Plant height	Stalk diameter	Total leaf number	Leaf area per plant	Ear fresh weight with husk	Ear fresh weight	Ear length	Ear diameter
D	91.1^{**}	80.9^{**}	47^{**}	16.3^{**}	1.46^{**}	325^{**}	34^{**}	11^{**}	9.8^{**}	1.7
H_1	70.1^{**}	65.1^{**}	426^{**}	17.9^{**}	2.87^{**}	558^{**}	246^{**}	174^{**}	22.9^{**}	61.7^{**}
H_2	54.9^{**}	48.5^{**}	373^{**}	12.9^{**}	2.33^{**}	488^{**}	222^{**}	174^{**}	19.7^{**}	59.9^{**}
h_2	196.8^{**}	204.1^{**}	$1,651^{**}$	48.3^{**}	10.41^{**}	$2,519^{**}$	$1,199^{**}$	537^{**}	107.8^{**}	227.7^{**}
F	53.0^{**}	42.1^{**}	-27	12.2^{**}	0.41	-50	-21	-8.5	6.9^{*}	0.1
E	3.1^{*}	3.6^{*}	4	0.6^{*}	0.38^{**}	11	30	1	0.1	1.1
$D-H_1$	20.9^{**}	15.8	-378^{**}	-1.5	-1.41^{*}	-233^{**}	-212^{**}	-163^{**}	-13.0^{**}	-60.0^{**}
$(H_1/D)^{1/2}$	0.8	0.8	2	1.0	1.40	1.3	2.68	3.88	1.52	5.98
$H_2/4H_1$	0.1	0.2	0.2	0.2	0.20	0.2	0.22	0.24	0.21	0.24
Kd/kr	1.9	1.8	0.8	2.1	1.22	0.8	0.78	0.99	1.60	1.01
h_2/H_2	3.584	4.203	4.42	3.718	4.45	5.1	5.38	3.0	5.45	3.79
$h^2(N)$	61.28	63.78	39.59	53.6	44.9	62.4	40.52	12.07	37.29	9.52
r	0.923^{*}	0.865^{*}	-0.90^{*}	-0.801^{*}	-0.74^{*}	-0.9^{*}	-0.91^{*}	-0.99^{*}	-0.95^{*}	-0.97^{*}

$^{*, **}$ Significant at the 5% and 1% probability levels, respectively.

期、吐絲期二性狀之 (Yr) 與 (Wr + Vr) 間關係為正值且顯著，表示控制這兩性狀的顯性為減量作用，即開花期與吐絲期愈早之品種，其顯性基因愈多，而開花期與吐絲期愈晚之品種，其隱性基因愈多。其它株高、稈徑、葉面積、含苞葉果穗重、去苞葉果穗重、穗長及穗徑等性狀之 (Yr) 與 (Wr + Vr) 間關係為負值且顯著，表示控制這兩性狀的顯性作用之方向為增量作用，即株高愈矮、稈徑愈小、葉面積愈小、含苞葉果穗重愈輕、去苞葉果穗重愈輕、穗長愈短、穗徑愈小之品種，其隱性基因愈多；反之，株高愈高、稈徑愈大、葉面積愈高、含苞葉果穗重愈重、去苞葉果穗重愈重、穗長愈長及穗徑愈大之品種，其顯性基因愈多。

討論

本試驗選 9 品種之自交系與其半互交之 F_1 為材料，計 45 組合，經 Hayman (1954a, 1954b) 之方法進行變方分析，全部性狀在組合間皆達極顯著差異。所有性狀在累加性作用上，雜交組合的顯性效果，平均顯性效果對顯性效果之影響，及顯性效果同時受到特殊組合力 (SCA) 的影響均呈顯著。開花期、吐絲期、株高、稈徑、葉面積、含苞葉果穗重及穗長等性狀之顯性效果同時受到親本所帶有之遺傳因子頻度不相等所造成。

Zeng *et al*. (2008) 指出超甜玉米花期性狀的遺傳符合加性—顯性模式。超甜玉米花期性狀的遺傳方差由加性效應和顯性效應共同組成，顯性效應比加性效應更為重要，即顯性基因起主要作用。控制花期性狀的基因至少有 3 對以上。超甜玉米花期性狀既有顯性基因效應，又有加性基因效應，其遺傳力較高，顯性基因效應大於加性基因效應。而本試驗顯示開花期及吐絲期受基因之累加性作用、基因之顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同控制，而其累加性效果大於顯性效果外，該性狀容易受環境影響。顯性作用大部分係受正負效應基因頻度分布不均造成的，其呈現顯性基因數目較多，控制著性狀之顯性基因群數有 3–5 群，其顯性基因為減量基因，且屬不完全顯性。本試驗結果與 Zeng *et al*. (2008) 之結論相似。

株高性狀受基因之累加性作用、顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同控制，顯性效果大於累加性效果、不易受環境影響。平均顯性程度為超顯性。該性狀

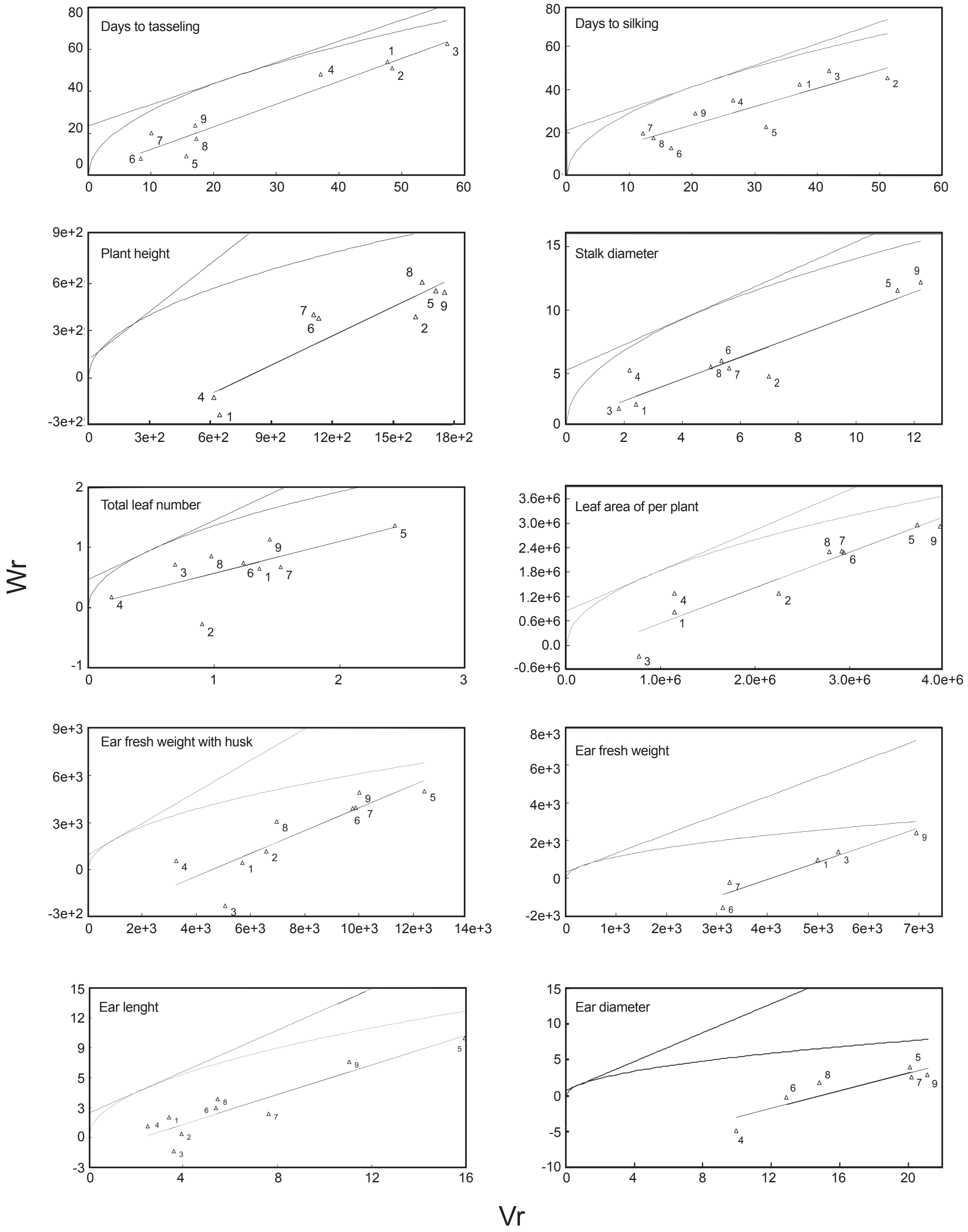

圖 1.　農藝性狀之 Wr/Vr 迴歸圖。1：PH 5-5、2：BJ12、3：PH10-23、4：SH-1、5：GH-8、6：Venus-8、7：Honey 236-1、8：HJ27、9：SN3-8。

Fig. 1.　Wr/Vr graphs of agricultural characters. 1: PH 5-5, 2: BJ12, 3: PH10-23, 4: SH-1, 5: GH-8, 6: Venus-8, 7: Honey 236-1, 8: HJ27, 9: SN3-8.

之顯性作用大部分是正負效應基因頻度分布不均所造成，其隱性基因數目較多。控制該性狀之顯性基因群數有 5–6 群基因控制，其顯性基因為增量作用，呈超顯性作用。其與 Shieh & Thseng (2002) 在台南白為材料進行株高與穗位高之全互交分析之結果雷同。

Ho *et al*. (1994) 在全互交分析報告中指出玉米稈徑性狀之表現呈現顯性效果大於累加性效果。而本研究以超甜玉米為材料，結果顯示稈徑性狀受基因之累加性作用、顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同控制。性狀之顯性效果大於累加性效果，容易受環境影響。平均顯性程度為完全顯性，顯性作用大部分是正負效應基因頻度分布不均所造成的，其顯性基因數目較多，有 3–4 群基因控制著性狀的表現，狹義遺傳率為 54%。其顯性基因為增量基因，呈完全顯性。

總葉數受基因之累加性作用、顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同影響，顯性效果大於累加性效果，不易受環境影響。顯性作用大部分係由正負效應基因頻度分布不均所造成的，顯性基因數目較多。有 4–5 群控制該性狀之表現，其顯性基因為增量基因，呈完全顯性。此結果與 Bonaparte (1977)、Wu (1987) 認為玉米葉片數明顯受累加性基因所控制，增加葉片數為部份顯性基因效應之結果稍有差異，可能係因材料與栽培環境不同，致使顯性程度表現不同所致。

葉面積性狀受基因之累加性作用、顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同影響，顯性效果大於累加性效果，不易受環境影響。顯性作用大部分是正負效應基因頻度分布不均所造成的，有 5–6 群基因控制該性狀之表現。其顯性基因為增量基因，呈超顯性作用。Zhou *et al*. (2008) 指出玉米葉面積主要受到顯性遺傳效應的影響。Wun *et al*. (2002)、Cal *et al*. (2002) 一致指出玉米葉片面積的遺傳基本上屬於數量遺傳。發育遺傳學認為數量性狀的形成涉及眾多基因的調控和相互作用，其遺傳表達與發育時期和環境條件有著密切的關係，即除了受遺傳主效應和環境效應的影響外，還受到基因型 × 環境互作效應的影響。本試驗顯示葉面積性狀受顯性影響效果大於累加性效果，與上述學者研究結果類似，易受環境影響。

本試驗顯示含苞葉果穗重與去苞葉果穗重受基因之累加性作用、顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同影響。顯性效果大於累加性效果，顯性作用大部分是正負效應基因頻度分布不均造成的，含苞葉果穗重其隱性基因數目較多；而去苞葉果穗重其顯隱性基因在親本的基因座上分布頻度均勻，控制性狀之顯性基因群數分別有 5–6 群與 3–4 群，其顯性基因為增量基因，呈超顯性作用。Malvar *et al*. (1996) 指出產量主要受顯性變方所影響。Xie & He (2003)、Wang *et al*. (2005) 以超甜玉米為材料，指出鮮穗重主要受顯性基因作用，且存在超顯性現象，顯性作用基因頻度分布不均，控制親本之顯性基因頻率大於隱性基因，而控制鮮穗產量的顯性基因至少有 4 對，並認為存在非等位基因的互補效應，顯性效應比加性效應重要。Shieh & Thseng (2002) 以食用玉米台南白為材料發現去苞葉果穗鮮重係由累加性及顯性效果共同控制，其中顯性效果是由基因平均顯性效果，頻度分布不均及特殊組合等共同控制，呈現基因顯性效果大於累加性效果，而顯性作用由 6 群超顯性基因所控制，並具增量效應。本試驗結果與之相似。

穗長及穗徑受基因之累加性作用、顯性作用、組合的顯性作用及所有雜交組合之顯性效果共同影響，性狀之顯性效果大於累加性效果。顯性作用大部分是正負效應基因頻度分布不均所造成的，即指顯隱性基因在親本的基因座上分布頻度不均勻。穗長其顯性基因數目較多；而穗徑其顯性基因數目相近。穗長及穗徑分別受 5–6 群與 3–4 群顯性基因控制性狀之表現，其顯性基因為增量基因，呈超顯性。本試驗顯示超甜玉米之結果與 Shieh & Thseng (2002) 指出食用台南白玉米之穗徑與穗長則受 6 群顯性基因控制性狀之表現，其顯性基因為增量基因之結果相似。

超甜玉米重要農藝性狀之遺傳介量與基因效應之分析，以 Hayman (1954a, 1954b) 方法分析結果，發現超甜玉米植株及果穗性狀係由

累加性與顯性效果共同控制，其中顯性效果係由基因平均顯性效果，頻度分布不均及特殊組合力等共同控制。超甜玉米植株及果穗鮮重等性狀若以 $D\text{-}H_1$ 的正負值為指標，則呈現基因顯性效果大於累加性效果，同時亦顯示超甜玉米含苞葉果穗鮮重之顯性作用係由 6 群超顯性基因所控制，其顯性基因均為增量基因。本試驗之結果與食用白玉米之遺傳現象相類似 (Shieh & Thseng 2002)。品種間組合力分析之結果，超甜玉米分為熱帶型與溫帶型相比較，顯示熱帶型帶有正的 GCA 效應者居多 (增量作用)，而溫帶型則為負的 GCA 效應者居多 (Liu *et al*. 2009)。本試驗由含苞葉果穗鮮重及去苞葉果穗鮮重之 Wr/Vr 迴歸圖形，也可看出由熱帶型而來之自交系 PH10-23、BJ12、SH-1 呈現帶有較多的顯性基因 (增量基因)；而溫帶型之自交系 Venus 8、GH-8、Honey236-1、SN 3-8 則帶有較多的隱性基因 (減量基因，或可進一步說明熱帶型之自交系 PH10-23、BJ12、SH-1 帶有延遲開花及吐絲，增加株高、穗位高、稈徑、葉數、單株葉面積、含苞葉果穗鮮重、去苞葉果穗鮮重、穗長與穗徑之效應。

由狹義遺傳率的估算，顯示各性狀之狹義遺傳率均小，介於 0.09–0.63 之間，係屬於數量遺傳之微效基因所控制。因此未來在超甜玉米之育種中，要將熱帶型與溫帶型的有利基因組合在一起，短期目標可直接利用熱帶型與溫帶型之自交系相互雜交，選出優質且高產之雜交組合，創立新品種；長期目標或須採用可組合含有較多有利基因的育種方法較為有利，如輪迴選種法進行熱帶型族群改良增加有利品質提昇之有利基因，通過熱帶型與溫帶型型間充分重組，不斷累積有利之累加性及非累加性基因，來創造更優異的熱帶型或溫帶型改良族群，或由此改良過的族群進行自交系之分離選拔，再經組合力檢定，選出具有組合熱帶型與溫帶型優點之 F_1 雜種供推廣栽培之用。

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Genetic Analysis on the Yield and Agronomic Characters of Super-Sweet Corn (*Zea mays* L.)

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Abstract

Liu, S. K. and G. J. Shieh. 2014. Genetic analysis on the yield and agronomic characters of super-sweet corn (*Zea mays* L.). J. Taiwan Agric. Res. 63(3):225–234.

This study used 9 maize inbreeds and 36 crossing combination (F_1) by half dialed to analyze the genetic background of yield and agronomic traits by the Hayman's method. The parents of 9 inbreds are 4 tropic and 5 temperate type. The agronomic traits included days to tasselings, days to silkings, plant height, ear height, stalk diameter, total leaf number, leaf area per plant, ear fresh weight with husk, ear fresh weight, ear length, and ear diameter. The results showed that vegetable characters were controlled by additive and dominant effects, and dominant effect was more important than additive effect. Average dominant effect of gene action was mostly overdominant, and there were 4–6 groups of dominant genes to control all the agronomic traits, and the dominant genes were the increasing effects. Days to tasseling and days to silking were co-controlled by additive and dominant effects, and additive effect was more important than dominant effect. Hence, those two traits were easily affected by environment, there were 3–5 dominant gene groups controlling the traits of days to tasseling and days to silking, and the dominant genes were the decreasing effect. Ear fresh weight with husk and ear fresh weight characters were controlled by additive-dominance gene, and dominant effect was more important than additive effect, which were controlled by 5–6 and 3–4 dominant gene groups, and they were overdominant. Ear length and ear diameter characters were controlled by additive-dominance gene, which were controlled by 5–6 and 3–4 dominant gene groups ,and dominant effect was more important than additive effect, and they were overdominant, and the dominant genes were the increasing effects.

Key words: Super sweet corn, Diallel cross, Genetic components, Gene effects.

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薑科作物遺傳歧異度之分析

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摘要

謝凱勛、林素汝、吳永培。2014。薑科作物遺傳歧異度之分析。台灣農業研究 63(3):235–248。

薑黃 (Turmeric)，學名 *Curcuma longa* L.，為薑科薑黃屬植物，在工業與香料上是重要的經濟作物。本試驗以 19 個薑科作物及高雄市旗山區收集之 20 個薑黃收集系為材料，探討薑科植物及薑黃收集系間之遺傳變異性與親緣關係，其中以簡單序列重複區間 (ISSR) 分子標誌探討 19 個台灣薑科作物親緣關係時，發現 19 個薑科作物之遺傳相似性介於 0.1–0.985 之間。以 ISSR 資料經主作標 (PCoA) 分析法之三維立體圖，可將 19 個薑科劃分成 6 個類群，類群的分布結果顯示可用 ISSR 解析薑科作物之親緣關係，並可據此有效建立台灣薑科作物的生態庫與遺傳基因池。又在 19 個薑科分析中，6 個不同來源之薑黃材料之 ISSR 分子標誌存在一定程度之變異，故進一步以高雄旗山區薑黃進行 ISSR 分子標誌分析。根據分析結果，旗山區薑黃遺傳相似性介於 0.797–0.986，利用主座標分析 (PCoA) 圖可將 20 個薑黃收集系分成 4 類群，並以 CNY12 與其他收集系的相似性最低。本研究結果顯示，台灣薑黃作物可能因為受到環境壓力與人為選拔，造成 DNA 分子層次存在相當程度之變異。

關鍵詞：薑黃、遺傳歧異度、簡單序列重複區間 (ISSR)。

前言

薑黃 (*Curcuma longa* L.) 為薑科薑黃屬之根莖植物，原產於亞洲南部的熱帶性草本植物，在食品工業及醫藥上是相當重要的作物。在食品工業中，其廣泛應用於罐裝飲料生產、烘培產品、乳製品、冰淇淋、酸奶、黃色蛋糕、柳橙汁、餅乾、爆米花的顏色、糖果、蛋糕糖衣、麥片、醬汁、明膠與咖哩粉等多項。在亞洲國家中，薑黃常用於食品添加劑、防腐劑與著色劑等 (Antunes & Araujo 2000; Cecilio-Filho *et al*. 2000)。此外，薑黃在醫藥應用上以薑黃素 (curcumin) 最受重視，係一個重要的色素化合物，可作為人體免疫缺陷 Human Immunodeficiency Virus Type1 (HIV-1) integrase 的抑制劑 (Mazumder *et al*. 1995)。薑黃又含有數個單萜與倍半萜化合物，如 zingiberene、ar-turmerone 及 turmerone 等 (Apisariyakul *et al*. 1995)，主要活性為驅風、抗脹氣、抗真菌與抗血小板劑 (Lee 2006)，可用於膽道疾病、厭食、咳嗽、糖尿病的傷口、肝臟疾病、風濕病與鼻竇炎等疾病治療 (Ammon *et al*. 1992)。

薑黃於南亞與東南亞，甚至中國、澳大利亞及南太平洋都有發現，尤以泰國、印度的多樣性最高，各區域中至少有 40 種存在，其次為緬甸、孟加拉、印尼及越南。薑黃為染色體組三元體的異交作物 (2n = 3x = 63)，可利用地下根莖進行無性繁殖 (Sasikumar 2005)。在大多情況下不易以雜交方式進行遺傳改良，因此薑黃育種工作主要為種原引進與營養系之選拔 (Ravindran 2007)。雖然薑黃遺傳育種上的改良先決條件主要為種原收集與鑑定，但相關

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研究報告甚少，大多數研究侷限以外表性狀表現進行基原鑑定 (Chaudhary *et al*. 2006)。然利用外表性狀之種原鑑定，由於可使用之形態特性有限，且形態數據與品質性狀如 curcumin、oleoresin、essential oil 等含量，易因不同環境影響致使性狀表現出現差異，很容易造成誤判現象。因此，利用 DNA 分子標誌取代外表性狀之基原鑑定，不但減少種原鑑定上的錯誤，更能降低薑科作物同物異名的現象。尤其在根莖作物種原的保存上，可因減少重複收集的數量，提高了無性繁殖庫的管理與效率。

近年來薑黃因保健及香料上之應用效益而受重視，其商業生產利用性大幅提升，因此利用有效率且準確之 DNA 分子標誌的基原鑑定技術，將攸關薑黃產業的發展。而分子標誌具有精準及快速的特性，可有效鑑定種原之分子遺傳變異，且鑑定結果不受環境影響。未來於薑黃品種、育種選拔及原生種的保存上，透過分子標誌之輔助，將可迅速提升種原之遺傳訊息及鑑定的準確性，確立可利用之薑黃種原及育種選拔親本。分子標誌為分子層次差異之遺傳標誌，其原理是利用一個基因、遺傳元素、DNA 片段或染色體片段等，來標記或追蹤其他基因、特性或個體 (Lin 2011)。依分子標誌種類，可區分為 RAPD (randomamplified polymorphic DNA)、AFLP (amplification-fragment length polymorphism)、ISSR (inter simple sequence repeats)、SSR (simple sequence repeat) 及 SNP (single nucleotide polymorphism) 等。不同於形態與生化標記，分子標誌不易受環境變動影響，可準確的表達出植物物種間之遺傳多樣性程度 (Cheng & Huang 2009; Thimmappaiah *et al*. 2009)。

台灣地處熱帶與亞熱帶氣候區，十分適合薑黃的生長與栽培，因此薑黃在台灣各地區皆有零星的栽培。且由於各地海拔、氣候變化、生育地環境差異及農民自行留種栽培等，經此長期之栽培繁殖過程，可能已造成薑黃產生遺傳變異。但截至目前為止，並未有確切證據可茲證明，故造成國產薑黃在親本選擇、育種及分類上均存在一定程度的困擾。薑黃已成為世界重要的香料及保健作物，在印度更是主要出口香料作物之一，具有相當高的產業潛力，值得台灣進一步開發與利用。

目前薑黃在台灣尚未有經濟栽培品種的命名與推廣，進行台灣薑黃遺傳變異之研究，結果將可提供未來台灣發展薑黃之重要基礎。本試驗收集台灣 19 個薑科作物，以 ISSR 分子標誌技術分析其遺傳上有無發生變異。並利用選育自 500 個高雄市旗山區蒐集之 20 個自然生長薑黃收集系為基礎材料，探討 20 個優良的收集系在 ISSR 分子標誌的分子層次上是否已有變異存在，從而建立 19 個薑科植物及 20 個收集系之分子遺傳資訊與親緣關係。本試驗資料將可釐清台灣薑科植物與旗山區薑黃的遺傳變異性，提供台灣薑科作物之種原鑑定、原生種及分離族群之遺傳變異等相關訊息，並可作為未來優化薑黃育種時之重要基礎。

材料與方法

19 個薑科植物

從台灣各地實地採集 19 個薑科植物之根莖，其根莖採集以單芽方式單株繁殖，合計蒐集了 30 個薑黃根莖芽體，並栽種於行政院農委會農業試驗所嘉義分所試驗田。待地上部成長至穩定狀態時，採集單株新鮮之幼葉進行 ISSR 分子標誌試驗，19 個薑科植物如表 1 所示。

高雄市旗山區薑黃收集系

本試驗材料以 2009 年自高雄市旗山區蒐集所得之 500 個薑黃根莖芽體為基礎，第一年每一薑黃根莖先進行單芽繁殖成單株，其後以目視方式選拔 100 個根莖體積較大者供下一年度繼續營養繁殖選拔。經二年選拔之後，共選出 20 個收集系，作為產量試驗及 ISSR 分子標誌分析用材料。

ISSR 分子標誌分析

DNA 萃取：採用 Li *et al*. (1995) 所發表的方法加以修改。將新鮮薑黃葉片進行冷凍乾燥處理後，剪取約 3 cm 葉片放入 1.5 mL 的防爆離心管中，加入 2 顆小鋼珠，置於組織研磨機中粉碎離心管中薑黃葉片。粉碎後將鋼珠取

表 1. 19 個薑科植物之材料採樣地點。
Table 1. Sampling sites of 19 Zingiberaceae plants.

Number	Scientific name	Chinese name	Location
1	*Curcuma longa*	屏東薑黃	Pingtung
2	*Curcuma longa*	台東薑黃	Taitung
3	*Curcuma longa*	名間薑黃	Nantou
4	*Curcuma longa*	東山薑黃	Tainan
5	*Curcuma longa*	嘉義薑黃	Chiayi
6	*Curcuma phaeocaulis*	嘉義莪朮	Chiayi
7	*Curcuma phaeocaulis*	屏東莪朮	Pingtung
8	*Curcuma phaeocaulis*	嘉義青薑 1	Chiayi
9	*Curcuma phaeocaulis*	嘉義青薑 2	Chiayi
10	*Zingiberzerumbet*	球薑	Chiayi
11	*Alpiniaofficinarum*	屏東南薑	Pingtung
12	*Alpiniaofficinarum*	台東南薑	Taitung
13	*Alpiniashimadae*	島田氏月桃	Chiayi
14	*Alpiniaspeciosa cv.Variegata*	斑葉月桃	Chiayi
15	*Zingiberofficinale Roscoe.*	竹薑	Chiayi
16	*Curcuma longa*	關仔嶺薑黃	Tainan
17	*Curcuma longa*	琉球薑黃	Chiayi
18	*Zingiberpurpureum Roscoe.*	紫薑	Chiayi
19	*Alpiniazerumbet*	大葉月桃	Chiayi

出，加入 300 µL 的 DNA 萃取緩衝液 (100 mM Tri-HCl, pH 9.0; 40 mM EDTA-2Na, pH 8.0; 20% SDS)，震盪均勻混合後，加入 150 µL Benzyl Chloride，震盪數秒。置於 50℃水浴鍋 15 min，加入 150 µL 的 3 M NaOAc，pH 5.2，置於 4℃下離心 21,380×*g* 15 min。取上清液於另一個新的離心管中，加入 800 µL 的異丙醇，置於 4℃下離心 21,380×*g* 10 min。除去液體，留下沉澱的 DNA，加入 500 µL 的 70% 乙醇洗鹽，除去液體風乾，再以 100 µL TE 緩衝液 (1 M Tris-HCl, pH 8; 0.5 M EDTA, pH 8.0) 回溶 DNA，於 4℃冰箱保存使用。

ISSR 分子標誌來源、PCR 及電泳分析： ISSR 引子使用加拿大哥倫比亞大學 (UBC801-900; University of British Columbia, Vancouver, British Coolumbia, Canada) 所設計之 100 組引子，參考 Zietkiewicz *et al*. (1994) 的方法，PCR 反應溶液總體積為 9 µL，分別為 10 ng μL^{-1} DNA、3.2 µL H_2O、5 mL *Taq* polymerase [150 mM Tris-HCl (pH 8.0)、1.5 mM $MgCl_2$、1% (w/v) 0.4 mM dNTPs]，於核酸增殖系統循環器 (sensoquest labcycler 96, Germany) 進行擴增。DNA 前期變性溫度為 94℃ 5 min，變性溫度 94℃ 1 min，引子鏈合溫度 50℃ 1 min，延展溫度 72℃ 1 min，重複變性至延展順序 35 次，最後再以 72℃ 5 min 將未完成延展 DNA 完成，最後結束在 20℃。PCR 擴增之 DNA 產物以 1.5% SFR (Amresco®, USA) 的洋菜膠，並添加追蹤染劑 (DNA loading dye 2 µL mL^{-1}) 與 100 bp DNA ladder (Gibco BRL)，利用高速電泳系統 MD-120 (Biokey-stone, USA)，在 150 V 持續 25 min 下進行電泳分析。完成的電泳膠片置膠體影像分析系統 (UV-tvausillumina) 做顯影紀錄。

資料分析

PCR 擴增後條帶出現有無以 0 與 1 表示，並計算所擴增出條帶總數與多型性條帶數。將所擴增出條帶以 Popgenever 1.31 (Yeh *et al*. 1997) 軟體計算族群間的相關遺傳資料，且

假設符合 Hardy-Weinberg equilibrium，來計算 Nei (1973) 遺傳歧異度指數 (H)：$1 - \sum_{i-1}^{N} p_i^2 p_i$ = 第 i 個等位基因頻率與 Shannon’s 訊息指數 (I) (Shannon & wiener 1963)，$I = -\sum P_i \log_2 P_i$，I 為表現型多樣性指數，Pi 為第 i 個等位基因頻率。

主座標分析 (Principal coordinate analysis; PCoA) 以 NTSYS-pc ver 2.0 所計算出之樣本間 Dice (1945) 相似度矩陣，進行 PCoA，以解釋變異比率最大的 3 個主成分作為主要座標軸，並繪製 2 維平面圖與 3 維立體圖，藉以了解收集系間於 3 維空間上的分布關係。

結果

薑科作物之遺傳歧異度

薑科作物多型性 ISSR 引子之篩選：本試驗使用加拿大哥倫比亞大學所設計之 100 個引子進行 ISSR 分子標誌分析，並從中選取擴增效果佳、條帶亮度強、具多型性與再現性佳的引子，結果共選定 13 個 ISSR-UBC 分子標誌，包括 UBC808、UBC809、UBC811、UBC817、UBC826、UBC828、UBC835、UBC836、UBC842、UBC847、UBC861、UBC868 與 UBC884 等。其中，UBC808 可擴增 19 條條帶為最高，其次為 UBC817 擴增 18 條條帶，而 UBC842 僅擴增 1 條條帶為最少。13 個引子用於 19 個薑科植物分析，共計擴增 152 條之 DNA 片段，其中 152 條條帶在 19 個薑科植物間均呈現多型性，各引子擴增具多型性條帶數目分別為 19、13、17、18、16、6、10、14、1、14、5、8 與 11。整體多型性百分比為 100 %，顯示本次所選擇 13 個引子用於 19 個薑科植物分類上具有不錯的效果 (表 2)。

薑科作物之遺傳歧異度與 Shannon’s 訊息指數分析：族群 P1 所包含的 19 個薑科作物 DNA 經 ISSR 標誌引子擴增後，產物再經電泳分析擴增條帶多型性之情形，據此資料進一步計算出收集系間的遺傳資訊，並將自交率指數 (Fis) 設定為 0，計算遺傳歧異度與訊息指數。經分析結果如表 3 所示，在族群 P1 薑科作物間可觀察到的等位基因數 (Na) 為 2.00，有效等位基因數 (Ne) 約為 1.61。在遺傳歧異度 (H) 中，其經軟體分析結果為 0.3582，Shannon 訊息指數為 0.5346。

薑科作物之親緣關係：薑科作物之親緣關係如表 4 所示，19 個薑科植物之遺傳相似性介於 0.1–0.985，由遺傳相似性之分布範圍十

表 2. 19 個薑科植物多型性 ISSR 引子之條帶擴增數、多型性條帶數量、多型性百分比與產物大小。

Table 2. The number of total amplified bands, polymorphic amplified bands, percentage of polymorphism and product size for 19 plants of Zingiberaceae by 13 ISSR polymorphic primers.

Primer	Total no. of amplified bands	No. of polymorphic bands	Percentage of polymorphic bands (%)	Product size (bp)
UBC808	19	19	100	300–2000
UBC809	13	13	100	250–1700
UBC811	17	17	100	380–1900
UBC817	18	18	100	280–1600
UBC826	16	16	100	380–1800
UBC828	6	6	100	550–1600
UBC835	10	10	100	300–2000
UBC836	14	14	100	380–1600
UBC842	1	1	100	1200
UBC847	14	14	100	300–2000
UBC861	5	5	100	550–1400
UBC868	8	8	100	300–1400
UBC884	11	11	100	300–1100
Total	152	152	100	

表 3. 兩個薑科族群透過 ISSR 條帶分析之遺傳多樣性。

Table 3. The genetic diversities through ISSR analysis among 2 populations of *Curcuma longa*.

Population	Na[z]	Ne	H	I
P1 (Zingiberaceae)	2.0000 ± 0.0000	1.6137 ± 0.2900	0.3582 ± 0.1254	0.5346 ± 0.1491
P2 (CNY)	1.5205 ± 0.5030	1.3595 ± 0.4015	0.2026 ± 0.2145	0.2960 ± 0.3057

[z] Na: Observed number of alleles, Ne: Effective number of alleles, H: gene diversity, I: Shannon's information index.

分寬廣來看，19 個薑科植物間有相似性極高者，亦有差異相當大者。其中，以薑黃屬中之屏東薑黃、台東薑黃、名間薑黃、東山薑黃與嘉義薑黃間相似性較高，其遺傳相似性介於 0.819–0.977，並以屏東薑黃與台東薑黃間之相似性最高，而琉球薑黃與其它 6 種薑黃間之遺傳相似度介於 0.283–0.327，顯示琉球薑黃與台灣的薑黃間之遺傳組成差異相當大。至於檢視薑黃與其它薑科作物間之親緣關係，結果可知薑黃與紫薑關係最近，其次為島田氏月桃，莪述、青薑、斑葉月桃、大葉月桃，而與台東南薑、屏東南薑、球薑及竹薑間之遺傳關係最遠；而在南薑分析中，結果顯示與球薑間之遺傳相似度最高，達 0.985；而與屏東薑黃間之遺傳關係最遠，相似度僅 0.1 而已，而與其它薑科間之遺傳相似度則介於 0.321–0.488；在月桃的分析中，除琉球薑黃與 3 種月桃間之遺傳相似度介於 0.294–0.349 外，其中以島田氏月桃和薑黃關係較相近，遺傳相似度介於 0.752–0.839，而其餘 2 種月桃與薑黃間之遺傳相似度僅介於 0.423–0.5，顯示月桃內有相當程度之分化，致使其與薑黃間之遺傳相似度高低差異頗大；至於莪述、青薑等、紫薑等與薑黃間之遺傳相似度亦較高，其次為球薑和南薑，而與月桃和竹薑間之親緣關係稍遠。在 19 種薑科作物遺傳相似度分析中其中最特別的為琉球薑黃，雖然其名稱為薑黃但與台灣各地所收集之薑黃相似度卻相對較低。

薑科作物 ISSR 多型性之主座標 (PCoA) 分析：以 19 個薑科植物為材料，藉由 13 組 ISSR 分子標誌分析後，所得數據計算出各薑科作物間的相似度矩陣，再進行主座標分析。PCoA 結果，第一維度 (Dim-1) 可以解釋 27.06% 變異程度，第二維度可以解釋 19.97% 變異程度，第三維度可以解釋 12.0% 變異程度，三維座標共解釋 59.04% 的變異程度；由圖 1 可以發現 19 個薑科植物散佈於圖的各位置。

第一維度於 -0.06 可將 19 個薑科植物分成兩群，又以第二維度於 -0.03 時，可將 19 個薑科植物再細分，故此從圖 1A 可以得知，19 個薑科植物可以分成 3 大群，第 1 群由屏東南薑、球薑、竹薑、斑葉月桃、大葉月桃、台東南薑與琉球薑黃組成，在此群中，屏東南薑與球薑距離較近；第 2 群由關仔嶺薑黃、紫薑、島田氏月桃、名間薑黃、嘉義薑黃、屏東薑黃、台東薑黃與東山薑黃組成，在此群中又以名間薑黃、嘉義薑黃、屏東薑黃、台東薑黃與東山薑黃距離較近；第 3 群由嘉義莪朮、屏東莪朮、嘉義青薑 1 與嘉義青薑 2 組成，在此群中，屏東莪朮、嘉義青薑 1 與嘉義青薑 2 距離相當近。

在三維立體圖之圖 1B 結果可以發現 19 個薑科植物分群，約介於 -0.53 至 0.50 間，與二維圖的結果相同，在第三軸 (Dim-3) 0.50 時，球薑與屏東南薑位於相同的高度；屏東莪朮、嘉義青薑 1 與嘉義青薑 2 等 3 者的高度也是相同；島田氏月桃、關仔嶺薑黃與紫薑的高度相同；名間薑黃、嘉義薑黃、屏東薑黃、台東薑黃與東山薑黃的高度也是相同。與大部分薑科植物高度相差最遠的為大葉月桃，位在 Dim-0.53。

由第三軸的數值可以得知，19 個薑科均位於不同的水平面上，但從二維可發現，19 個薑科能夠明顯的分成 3 群，由結果又可以得知，大葉月桃與其他薑科差異較大。

薑黃收集系之遺傳歧異度

薑黃收集系 ISSR 分子標誌多型性：利用 100 個 ISSR 引子於薑黃收集系進行篩選，從中篩選擴增效果佳、條帶亮度強，具多型性且再現性佳之引子，結果共篩選出 11 組 ISSR-UBC 分子標誌，包括 UBC807、UBC808、

表 4. 19 個薑科植物 ISSR 基因型的相似性係數。

Table 4. Dice coefficients of similarity based on ISSR data among 19 Zingiberaceae plants.

Item[z]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-																		
2	0.977	-																	
3	0.962	0.985	-																
4	0.969	0.961	0.947	-															
5	0.953	0.961	0.977	0.969	-														
6	0.650	0.645	0.634	0.650	0.633	-													
7	0.545	0.541	0.531	0.564	0.545	0.804	-												
8	0.600	0.595	0.585	0.600	0.583	0.929	0.843	-											
9	0.600	0.595	0.585	0.600	0.583	0.929	0.863	0.982	-										
10	0.391	0.388	0.412	0.375	0.391	0.417	0.327	0.400	0.417	-									
11	0.100	0.397	0.421	0.385	0.400	0.41	0.321	0.393	0.410	0.985	-								
12	0.375	0.372	0.365	0.375	0.375	0.404	0.34	0.365	0.365	0.411	0.421	-							
13	0.784	0.778	0.797	0.752	0.784	0.564	0.523	0.547	0.564	0.480	0.488	0.349	-						
14	0.459	0.473	0.482	0.477	0.495	0.396	0.374	0.376	0.376	0.477	0.468	0.387	0.509	-					
15	0.379	0.393	0.387	0.362	0.362	0.370	0.184	0.333	0.333	0.448	0.458	0.380	0.319	0.412	-				
16	0.819	0.813	0.831	0.787	0.819	0.588	0.514	0.555	0.555	0.472	0.481	0.36	0.839	0.500	0.383	-			
17	0.286	0.283	0.296	0.286	0.305	0.371	0.322	0.351	0.351	0.381	0.374	0.315	0.294	0.349	0.344	0.327	-		
18	0.882	0.859	0.877	0.850	0.866	0.605	0.514	0.555	0.555	0.441	0.450	0.342	0.893	0.481	0.365	0.937	0.288	-	
19	0.423	0.438	0.430	0.442	0.442	0.396	0.302	0.313	0.333	0.308	0.321	0.455	0.416	0.518	0.348	0.427	0.296	0.408	-

[z] The number of materials is the same as in Table 1.

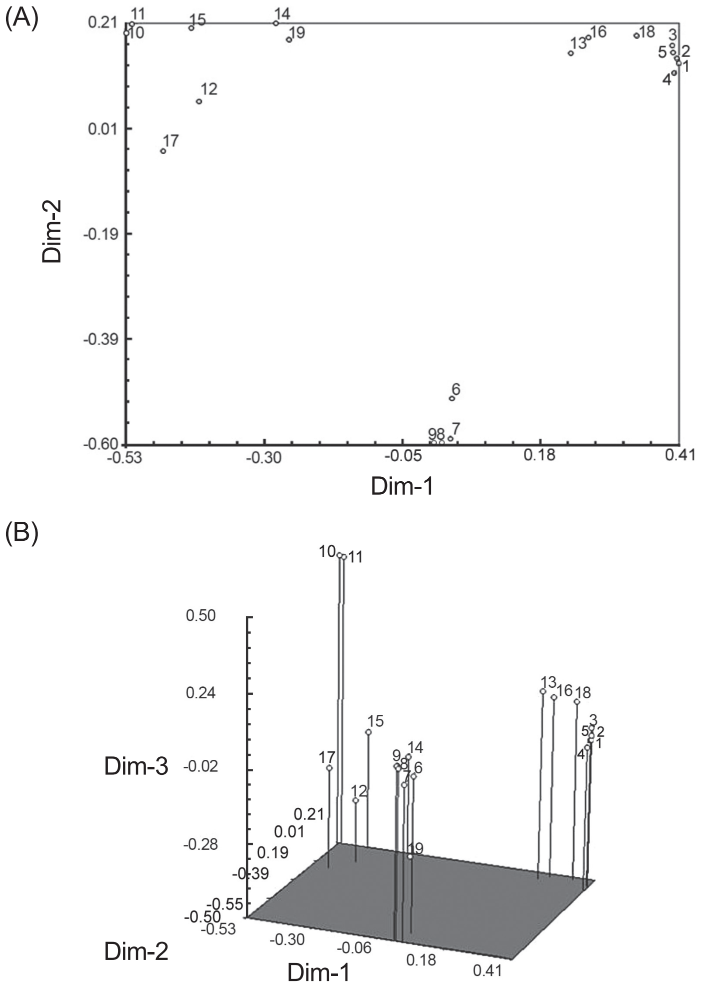

圖 1. 19 個薑科植物之 ISSR 資料的主座標分析圖。

Fig. 1. The principal coordinates analysis map based on ISSR data for 19 Zingiberaceae plants. The number of materials are same as in Table 1.

UBC810、UBC825、UBC826、UBC835、UBC836、UBC841、UBC847、UBC861及UBC880 等，11 組引子分別擴增出 3、11、8、5、8、4、6、9、5、7、8 條條帶。其中，以 UBC808 擴增出 11 條條帶最高，而 UBC807 擴增 3 條條帶最少。11 個引子用於 20 個收集系分析，共擴增出 74 條 DNA 片段，其中有 36 條條帶在 20 個收集系間呈現多型性，具多型性引子中各擴增條帶數目分別為 2、1、7、4、1、1、5、5、2、3、5。以 UBC810 可擴增出 7 個多型性條帶最高，而 UBC808、UBC826 及 UBC835 僅可擴增出 1 個條帶最少，整體而言多型性條帶所占百分比為 49 % (表 5)。

薑黃收集系遺傳歧異度與 Shannon's 訊息指數分析：族群 P2 所包含的 20 個收集系 DNA 經 ISSR 標誌引子擴增後，以其擴增出條帶，記錄出現的多型性，計算出收集系間的遺傳資料 (Yeh *et al*. 1997)，將自交率指數設定為 0，計算遺傳歧異度與訊息指數。經分析結果如表 3，在族群 P2 的薑黃收集系間可觀察到的等位基因數為 1.52，有效等位基因數約為 1.36。在遺傳歧異度中，其經軟體分析結果為 0.20，Shannon 訊息指數為 0.29。

表 5. 20 個薑黃收集系 ISSR 多型性引子序列之條帶擴增數、多型性條帶數量、多型性百分比與產物大小。

Table 5. The number of total amplified bands, polymorphic amplified bands, percentage of polymorphism and product size for 20 collections of *Curcuma longa* L. by analysis of 11 ISSR polymorphic primers.

Primer	Total no. of bands	No. of polymorphic bands	Percentage of polymorphic bands (%)	Product size (bp)
UBC807	3	2	67	350–1200
UBC808	11	1	9	350–1500
UBC810	8	7	88	400–1800
UBC825	5	4	80	480–1500
UBC826	8	1	13	390–1700
UBC835	4	1	25	300–1300
UBC836	6	5	83	500–1000
UBC841	9	5	56	290–1900
UBC847	5	2	40	300–1600
UBC861	7	3	43	500–1600
UBC880	8	5	63	310–1300
Total	74	36	49	

薑黃收集系遺傳相似性分析：20 個薑黃收集系之遺傳相似性介於 0.797–0.986，除收集系 CNY12 與 CNY6 的相似度 0.797 外，其餘大部分收集系間之相似性在 0.8 以上。在 20 個收集系中，其中收集系間遺傳相似性介於 0.0.8–0.85 者有 15 個，介於 0.851–0.90 者有 37 個，而介於 0.901–0.95 者有 101 個，而相似性在 0.95 以上者則有 36 個 (表 6)。由遺傳相似性分析可知大部份薑黃收集系均十分相近，其中相似性在 0.90 以上者約 137 個，顯示旗山區薑黃收集系間之親緣關係十分密切；這些收集系中除了 CNY6 與 CNY3 間相似性較低外 (0.843)，以 CNY12 與 CNY6、CNY8、CNY17、CNY2、CNY7、CNY11、CNY1、CNY15、CNY14、CNY9、CNY19、CNY20、CNY5、CNY13、CNY18 等的相似性較低，相似性分別為 0.797、0.807、0.81、0.826、0.826、0.826、0.828、0.829、0.831、0.843、0.843、0.845、0.847、0.85、0.85、0.85，是親緣關係較遠者。至於相似性較高者如收集系 CNY13 與 CNY2、CNY9、CNY14 與 CNY2、CNY18 與 CNY7、CNY18 與 CNY9、CNY15 與 CNY13、CNY18 與 CNY16 間的遺傳相似度數值均為 0.978；而 CNY15 與 CNY9 間的相似性為 0.986，是所有收集系間遺傳相似性最高者，顯示親緣關係最為相近。

薑黃收集系 ISSR 多型性資料之主座標 (PCoA) 分析：以 20 個薑黃收集系為材料，藉由 ISSR 分子標誌分析後，所得數據計算出各收集系間的相似度矩陣，再進行 PCoA 分析。PCoA 結果顯示，第一主成分可以解釋 24.68% 變異程度，第二主成分可以解釋 12.97% 變異程度，第三主成分可以解釋 9.83% 變異程度，三個主成分共解釋 47.47% 的變異程度。

在三個主成分所繪製之二維立體圖中，其第一維度於 -0.1420 劃分 20 個收集系，20 個收集系顯然較為分散，但是在第一維度約等於 0.01 與第二維度約等於 0.066 可劃分出 1 群，為 CNY04、CNY11、CNY02、CNY09、CNY08、CNY06、CNY13、CNY01、CNY14、CNY15、CNY07 與 CNY18。其中在第一維度約等於 0.13 上，CNY08 與 CNY06 最近，其次為 CNY15 與 CNY07，2 者為 2 個更小 1 群。在第一維度約等於 -0.01 與第二維度約等於 -0.12 上，有 CNY16、CNY19、CNY17 與 CNY20 為 1 群。CNY03、CNT10、CNY12 與 CNY05，不屬於這 2 群中，較分散於圖中，並以 CNY12 與其他收集系的相對距離較遠 (圖 2A)。

在三維立體圖結果可以發現 20 個收集系分群，介於第三維度約等於 0.14 至 -0.42 間，

表 6. 20 個薑黃收集系之 ISSR 基因型的相似性係數。

Table 6. Dice coefficients of similarity based on ISSR showing the relationship between 20 *Curcuma longa* L. collected lines.

	CNY01	CNY02	CNY03	CNY04	CNY05	CNY06	CNY07	CNY08	CNY09	CNY10	CNY11	CNY12	CNY13	CNY14	CNY15	CNY16	CNY17	CNY18	CNY19	CNY20
CNY01	-																			
CNY02	0.947	-																		
CNY03	0.891	0.887	-																	
CNY04	0.946	0.955	0.900	-																
CNY05	0.928	0.938	0.931	0.921	-															
CNY06	0.908	0.933	0.843	0.916	0.882	-														
CNY07	0.947	0.957	0.887	0.940	0.938	0.933	-													
CNY08	0.931	0.941	0.869	0.924	0.906	0.917	0.971	-												
CNY09	0.932	0.957	0.887	0.940	0.923	0.933	0.971	0.971	-											
CNY10	0.896	0.923	0.897	0.905	0.918	0.866	0.892	0.891	0.923	-										
CNY11	0.932	0.971	0.871	0.955	0.908	0.919	0.957	0.956	0.971	0.923	-									
CNY12	0.828	0.826	0.897	0.855	0.850	0.797	0.826	0.807	0.843	0.867	0.826	-								
CNY13	0.955	0.978	0.894	0.962	0.946	0.940	0.964	0.948	0.978	0.930	0.964	0.850	-							
CNY14	0.938	0.978	0.876	0.947	0.929	0.924	0.948	0.932	0.948	0.913	0.948	0.831	0.970	-						
CNY15	0.933	0.957	0.873	0.941	0.924	0.949	0.971	0.957	0.986	0.909	0.957	0.829	0.978	0.949	-					
CNY16	0.929	0.955	0.881	0.922	0.935	0.899	0.939	0.923	0.939	0.903	0.924	0.852	0.962	0.961	0.940	-				
CNY17	0.906	0.917	0.857	0.899	0.912	0.892	0.947	0.916	0.932	0.880	0.917	0.810	0.924	0.923	0.948	0.945	-			
CNY18	0.924	0.949	0.878	0.932	0.930	0.925	0.978	0.948	0.978	0.899	0.949	0.850	0.971	0.955	0.978	0.947	0.955	-		
CNY19	0.922	0.932	0.891	0.899	0.928	0.908	0.947	0.916	0.932	0.912	0.902	0.845	0.939	0.938	0.948	0.945	0.953	0.955	-	
CNY20	0.927	0.906	0.860	0.887	0.900	0.880	0.906	0.889	0.906	0.883	0.891	0.847	0.929	0.912	0.908	0.934	0.911	0.913	0.911	-

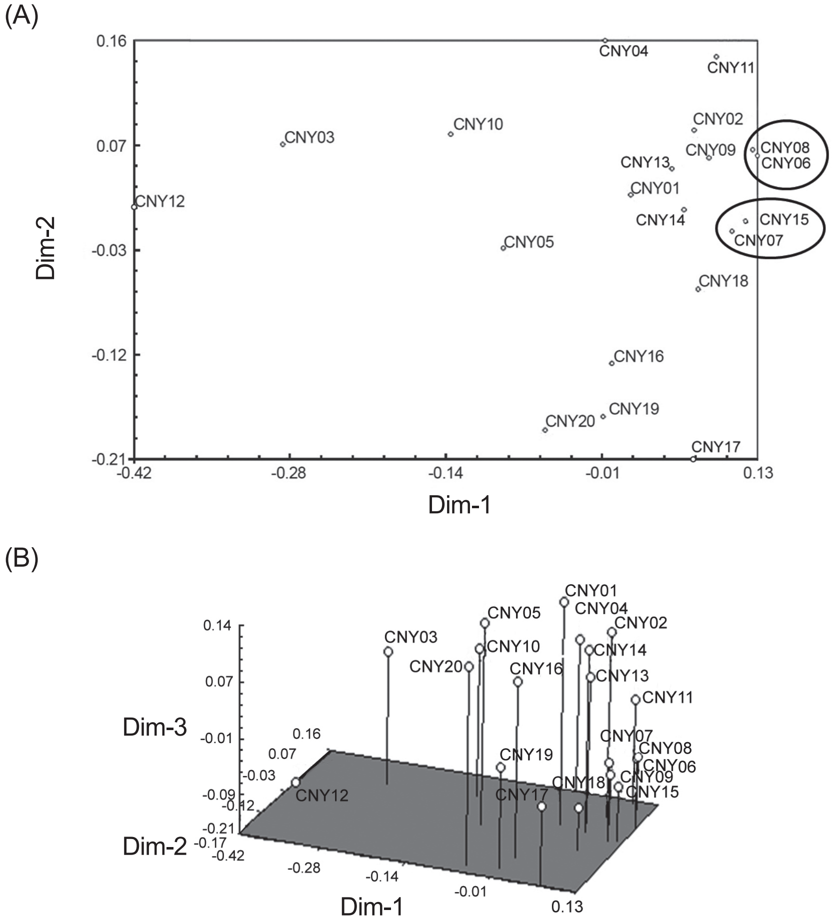

圖 2.　20 個薑黃收集系之 ISSR 資料的主座標分析圖。(A) 二維圖；(B) 三維立體圖。

Fig. 2.　Principal coordinates analysis map for ISSR markers of 20 *Curcuma longa* L. collected lines. (A) Two-dimension; (B) Three-dimension.

大部分收集系之高度差不多，但在 CNY12、CNY19、CNY11、CNY09、CNY17、CNY18、CNY07、CNY08、CNY06 與 CNY15 高度較其他收集系低，又以 CNY12 最低。CNY06 與 CNY08 兩個收集系間較接近。由第三座標軸的數值可以得知，20 個收集系之間的高度，均位於不同的水平面上，顯示 20 個收集系中存在著隱藏的變異，又以 CNY12 與其他收集系差異較大，其餘的收集系之分布則較為集中，可以約分成 4 群，即 CNY16、CNY17、CNY19 與 CNY20 為 1 群；CNY01、CNY02、CNY04、CNY14、CNY13、CNY11、CNY18、CNY06、CNY08、CNY09、CNY15 與 CNY07 為 1 群；CNY03、CNY05 與 CNY10 為 1 群；而 CNY12 則單獨 1 群 (圖 2B)。

討論

基因遺傳歧異度值越高，其調查的物種就會越豐富。19 個薑科植物之遺傳歧異度值為 0.358，Shannon’s information index 為 0.535，顯示 19 個薑科植物之基因歧異度相當高，其原因係 19 個薑科植物在科分類之下尚有屬及種，因屬及種下之各植物間差異甚大，故在遺傳歧異度上差異相當大是可預期之結果，此乃造成本次分析結果的遺傳歧異度及 Shannon’s

information index 值有較高的表現值。而由圖 1 之主成分三維立體圖得知 13 個 ISSR 分子標誌之分析已將薑科各屬間區分開，顯示可藉由 ISSR 分子標誌解析薑科作物之遺傳相似性，從而了解其親緣關係之遠近。

在所蒐集的台灣 19 個薑科作物的分析中，其中如屏東薑黃、台東薑黃、名間薑黃、東山薑黃、關仔嶺薑黃與琉球薑黃，在 ISSR 分子標誌親緣鑑定的分析中，其中以琉球薑黃與台灣各地區之薑黃相似性最低。推測其原因，可能是琉球薑黃為琉球當地農民自行篩選出之地方種，其選出之薑黃係經琉球環境、氣候及人為選拔之影響，由於琉球環境影響的因子與台灣地區不同，且人為選拔方式可能也與台灣不同，其也有可能為不同物種，故與台灣薑黃的相似度僅 0.32 (表 4) 是可理解之結果。

在 19 個薑科親緣分析結果中，由表 4 中發現台東薑黃與名間薑黃相似性高達 0.98，此在主座標分析圖 (圖 1) 中亦同樣明顯可見，兩者的距離極近且高度也相同。可以推斷，此二者為差異極小之薑黃，可能是農民以相同種原薑黃在台東與名間地區栽培散布所致。而嘉義青薑 1 及嘉義青薑 2 則為在嘉義不同地區所蒐集之青薑，試驗結果亦發現兩者間之遺傳相似性相當高，故此推測兩者亦為來源相近且相似度高的青薑族群。在月桃部分，島田氏月桃、斑葉月桃與大葉月桃等三種月桃的分析中，雖三者均為月桃屬，然三者之外表性狀差異相當大。此次試驗結果亦證明三者的遺傳相似性確實不高，其中以島田氏月桃與另外二者遺傳相似度最低，而斑葉月桃與大葉月桃的相似度較島田氏月桃高。又試驗結果另顯示屏東南薑與球薑間之相似性較台東南薑高，可能原因係屏東南薑與台東南薑間發生了變異或分化，故造成屏東南薑與台東南薑間之相似度反較之屏東南薑及球薑間相似度低的結果。

由 19 個薑科作物 ISSR 分子標誌分析結果得知，5 個台灣薑黃種間存在遺傳變異，顯示雖薑黃主要以營養繁殖為主，然經台灣地區多年的栽培與繁殖，其在 DNA 層次上已有遺傳變異存在。此由高雄市旗山區 20 個薑黃收集系之 ISSR 分子標誌分析結果證明，確實存在相當的遺傳變異。由於旗山區薑黃存在相當之遺傳變異，未來吾人當可利用台灣薑黃族群進行純化及選拔，藉此選育適合台灣栽培利用之薑黃新品種。

在 20 個薑黃收集系中已有 11 個引子表現出多型性，11 個引子所擴增之多型性條帶亦有 36 條，這些多型性分子標幟應已足供釐清 20 個薑黃收集系間遺傳距離利用，而 11 個 ISSR 引子中，UBC810、UBC825 與 UBC836 的多型性百分比分別高達 88%、80% 與 83%，顯示未來可利用這 3 個引子區分或判別薑黃品種間之差異性，未來可於薑黃品種間親緣鑑定或品種判別上利用。又 Gutierrez *et al*. (2005) 報告指出 ISSR 於整個基因組分布廣泛且豐富，較其他遺傳標誌有更高比率的多型性存在，而 Varshney *et al*. (2002) 亦指出 ISSR 分子標誌在基因組中特定的側翼序列微衛星位點，保留屬間甚至整個相關屬間、種內與不同種間之序列，印證本次試驗發現 3 個引子可於薑黃收集系種間之種原鑑定或遺傳相似度上探討利用之結論。

Cheng (2003) 應用 ISSR 探討玉山杜鵑 (*Rhododendron pseudochrysanthum* Hayata) 之族群遺傳變異研究，Nei's 基因歧異度為 0.3371；Ho *et al*. (2007) 以 ISSR 分子標誌探討阿里山十大功勞 (*Mahonia oiwakensis* Hayata) 族群遺傳多樣性，族群的 Nei's 基因歧異度 0.3762，在台灣裸子植物之刺柏 (*Juniperus formosana* Hayata) 基因歧異度 (Tsai 2004) 及唐杜鵑 (*Rhododendron simsii* Planch) 族群基因歧異度為 0.3423 (Chen 2005)；Ju & Wing (2008) 使用 ISSR 分子標誌檢測台灣鄰近地區的樟樹族群遺傳結構，台灣地區樟樹的基因的遺傳歧異度介於 0.068–0.208。這些相關研究結果顯示，由於台灣地區因環境存在一定壓力，致使所存在之物種發生了一定程度的族群分化。台灣薑科作物間遺傳歧異已達 0.3582，且旗山區薑黃收集系之遺傳歧異度亦達 0.2026，顯示台灣薑科或薑黃族群亦因環境存在壓力，使得這些長久在野地生長繁殖之薑科及薑黃，因受到當地環境壓力影響，結果因累積並產生一定程度之遺傳變異，故造成族群內已有分化的現象存在。Hamrick *et al*. (1992)

利用 isozyme 分析木本植物的數據進行統計，顯示蟲媒花異交植物之 Nei's 基因歧異度值平均為 0.211，特有種到廣泛分佈種之 Nei's 基因歧異度值為 0.078–0.257。He (2013) 利用 ISSR 分子標誌進行澎湖香茹 (*Glossogyne tenuifolia* Cass.) 族群間之比較，12 個族群中之 Beiliao 與 Fenggui 地區香茹族群遺傳歧異度為 0.215 及 0.204。由此得知，薑黃雖然為無性繁殖作物，但旗山地區薑黃的遺傳歧異度與異交作物相近，可見旗山區薑黃具有高度的遺傳變異。

在 Shannon's information index 中，當指數數值為 0 時，表示只有單一族群存在，本次測定結果為薑科作物為 0.5346 (表 3)，旗山區薑黃為 0.296 (表 6)，結果顯示台灣薑科作物與旗山區薑黃之 Shannon 訊息指數值均相當高，顯示在台灣薑科作物與旗山區薑黃可能受地理環境壓力之關係，因此可能有超過 1 個以上的薑科與薑黃族群存在。尤其對照 Ju & Wing (2008) 及 He (2013) 使用 ISSR 分子標誌檢測樟樹及澎湖香茹族群遺傳結構試驗結果，其中大陸樟樹的 Shannon's information index 平均為 0.313，台灣地區為 0.326；而在 12 個香茹族群中，Beiliao 與 Fenggui 之遺傳歧異度為 0.215 及 0.204，相較於本試驗 Shannon's information index 數值，顯示台灣薑科作物與旗山區薑黃 Shannon's information index 接近或高於大陸地區樟樹族群及澎湖香茹族群的分析結果。因此，可以推測台灣地區與旗山區的環境變化因子較大，致使台灣薑科作物及旗山區薑黃之 Shannon's information index 數值相對較高，故不論台灣薑科作物或旗山區薑黃應均存在 1 個以上的分離族群。

旗山區薑黃 Dice 的相似性分析結果介於 0.797–0.986，而數值越接近則相似性就越高，其中以 CNY12 與 CNY06 為 0.797。可見兩收集系之薑黃遺傳上差異最大，即可能在遺傳上較不相似，尤其 CNY12 與其他收集系之相似度均相對較低，顯示可能係遺傳發生變異或是環境的壓力造成 CNY12 與其他收集系相似度較低。CNY15 與 CNY09 相似度最高，對照於 PCR 擴增的條帶，在所有條帶中，兩收集系僅 1 個條帶呈現不同，故可將此條帶開發做為薑黃收集系的專一分子標誌，應用於薑黃品種之鑑定。而就旗山地區薑黃之主座標分析圖來看，旗山區薑黃可大致區分成 4 個類群 (圖 2)，其中 CNY12 與其他薑黃距離較遠，推測 CNY12 可能自不同地區所繁殖的薑黃或雜交植株，最後種植在旗山地區，所以造成相似性與主標分析和其他收集系薑黃相似性較低。在圖 2 中，CNY09 與 CNY15 兩者相似性最高，也就是其遺傳歧異度低，可能是在個體上有發生遺傳變異，或在 ISSR 分子標誌誤判，而造成相似度高。

本試驗結果可以發現，相同物種種植於不同地區，經過不同環境選拔壓力下，其 DNA 分子層次會出現差異，而由於 DNA 分子標誌不會受環境影響，因此不會造成分類上的錯誤。據此，未來國內薑科種保存及品種鑑定時，應可利用本試驗分子標誌分析結果，減少以外表性狀誤判種原或重複收集種原之錯誤，更可利用薑黃 ISSR 分析結果，針對台灣地區薑黃進行收集系或品種進行鑑別利用。尤其本試驗證明台灣地區薑黃在分子層次上已有存在相當之遺傳變異，在有遺傳變異育種純化與選拔方有效益的前提下，未來吾人可針對此一高經濟價值的作物，進行相關經濟性狀之評估，諸如根莖產量、類薑黃素 (curcumin)、總酚、抗氧化力及清除自由基能力等特性，找出產量佳且活性成分高之薑黃收集系進行經濟栽培，最後再透過分子標誌技術協助優良收集系或新品種純度的維持，奠定及擴大台灣薑黃商業生產的利基。

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Genetic Diversity of Zingiberaceae Crop

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Abstract

Hsieh, K. H., S. J. Lin, and Y. P. Wu. 2014. Genetic diversity of Zingiberaceae crop. J. Taiwan Agric. Res. 63(3):235–248.

Turmeric (*Curcuma longa* L.) is a member of ginger family, Zingiberaceae, and has become an important economic crop for industrial uses and spice production. In this study, a total of 19 ginger species and 20 turmeric collected lines which were gathered from Cishan area, Kaohsiung, Taiwan, were subjected to assess the genetic diversity and phylogenentic relationship by using inter-simple sequence repeats (ISSR) markers. For the 19 ginger species, the genetic similarity was ranged from 0.1–0.985, and six distinct groups were clustered by the results of the principal co-ordinates analysis (PCoA) and dendrogram. For the 20 turmeric lines, the genetic similarity was ranged from 0.797 to 0.986, and four distinct groups were clustered by the results of PCoA and dendrogram. Moreover, six of the 19 ginger species exhibited some genetic diversity revealed by ISSR markers. Among these 20 collected lines, CNY12 was exhibited phylogenetically far-related to the other collected lines. The genetic relationships of Zingiberaceae plants constructed herein offered an effective establishment of an agro-climate and a gene pool library of *C. longa* L. in Taiwan. Despite exposure to environmental stresses and artificial selection for a long time, the vegetative propagation of Taiwanese turmeric existed genetic diversity at DNA molecular level.

Key words: *Curcuma longa* L. (turmeric), Genetic diversity, Inter-simple sequence repeat (ISSR).

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東方果實蠅 [*Bactrocera dorsalis* (Hendel)] 乾式餌劑之耐雨及誘效持續性研究

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摘要

董耀仁、陳健忠。2014。東方果實蠅 [*Bactrocera dorsalis* (Hendel)] 乾式餌劑之耐雨及誘效持續性研究。台灣農業研究 63(3):249–252。

東方果實蠅 [*Bactrocera dorsalis* (Hendel)] 是台灣重要蔬果害蟲。本研究以連續浸水方式模擬下雨情況，測試東方果實蠅乾式餌劑耐雨性及經浸水後對毒效的影響。另將乾式餌劑懸掛於戶外，經不同時間後移回室內，測試其對東方果實蠅之誘殺持效性。試驗結果顯示，東方果實蠅乾式餌劑經浸水 8 h 後尚殘留約 50% 量的餌劑，且不影響對東方果實蠅毒效。餌劑懸掛於室外 8 wk 後誘殺效果維持不變。上述試驗證實乾式餌劑具有耐雨及誘效持久之特性，可應用於田間綜合防治東方果實蠅體系中。

關鍵詞：東方果實蠅、餌劑、耐雨性、果實蠅防治、害蟲綜合管理。

東方果實蠅 [*Bactrocera dorsalis* (Hendel)] 雌蠅產卵於果實內，孵化後幼蟲蛀食果肉影響水果產量和品質。本害蟲於水果輸出時被世界各國認為是重要的檢疫害蟲，常形成貿易障礙 (Chen *et al*. 2001)。針對東方果實蠅，目前以化學防治、含毒甲基丁香油滅雄、點噴含毒蛋白質水解物、果實套袋和清除被害果為主要的防治方法 (Lee 1988; Liu 1991)。其中點噴含毒蛋白質水解物，為政府推薦用於緊急防治東方果實蠅的方法，惟若遇雨水沖刷則完全失去誘殺效果，農民常因此需要補噴增加生產成本 (Chen *et al*. 2001)。Ye & Liu (2007) 研究指出，在適溫條件下，每月降雨量低於 50 mm，東方果實蠅族群密度降低，在 100–200 mm 反而有利東方果實蠅族群增加，而雨量大於 250 mm，則東方果實蠅數目顯著減少，顯示降雨是影響東方果蠅族群變動的重要因子。Teixeira *et al*. (2009) 將 16.7% GF-120 (賜諾殺餌劑) 25 μL 滴於蘋果葉上，於溫室內經 24 h 乾燥後，經 2.5 mm 模擬雨量沖刷 5 min 後，其對蘋果果實蠅雌蟲殺蟲效果與無農藥對照組無差異。由上述結果可知，累積降雨量與東方果實蠅的族群變動有關，但每次降雨均可能沖刷或稀釋已噴施之含毒蛋白質水解物並影響其誘效。因此，提高含毒蛋白質水解物等食物餌劑耐雨能力為學者研究重點。

國外已有研究可增加食物餌劑耐雨性方法，包括增加餌劑的施用濃度、將餌劑噴施或置於防雨資材、於餌劑中加入保濕劑，如澱粉等形成膠狀餌劑及於餌劑中加入不溶於水的石蠟等 (Chen *et al*. 2001; Mangan & Moreno 2007; Pinero *et al*. 2009; Texeira *et al*. 2009)。為了解決因雨水沖刷造成食物餌劑誘殺效果喪失的情形，本研究參考 Chen *et al*. (2010) 研發之瓜實蠅乾式誘殺錠，製成東方果實蠅乾式餌劑 (錠劑，直徑 3.0 cm，厚度 0.8 cm，重量 10.0 g)，除含有東方果實蠅取食誘引成分及農藥用以誘殺果實蠅外，並添加高吸水性高分子

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(superabsorbent polymers; SAP) 以提高餌劑耐雨能力。於先前室內浸水試驗中，未添加 SAP 餌劑經浸水 3 h 後，完全崩解溶於水中，而添加 SAP 錠劑經浸水 3 h 後，將錠劑取出置於 60℃烘箱經 3 d 後，取出秤重並計算餌劑流失率，與未浸水前重量相較僅流失 15.0%。而於戶外試驗中，將乾式餌劑於農試所試驗田區懸掛 14 d，在無任何遮蔽且於試驗期間每日雨量分別為 0.0、7.5、14.5、0.5、62.5、2.0、0.0、1.0、0.5、0.0、4.5、7.0、10.5 及 0.0 mm (行政院農業委員會農業試驗所氣象站資料，2010 年 8 月 21 日 – 9 月 3 日) 的情況下，未添加 SAP 對照組餌劑，因雨水沖刷完全消失，而有添加 SAP 餌劑者，流失率為 45.4% ± 1.3% (未發表資料)。由上述室內及室外初步試驗顯示，添加 SAP 會增加乾式餌劑之耐雨能力。

為測試此乾式餌劑之耐雨性，以及遇水後對東方果實蠅毒效影響與誘殺持效性共進行二組試驗。(一) 浸水模擬降雨對乾式餌劑流失與毒效影響試驗是將乾式餌劑放置於 250 mL 透明塑膠杯中後，加入 200 mL 水，分別經 1、2、3、4、5、6、7、8、16 及 24 h 後將殘留餌劑取出。一部分置於 60℃烘箱中經 3 d 後，取出秤重並計算餌劑流失率，另一部分於室內陰乾 24 h 後備用。將 10 對 (已饑餓 16–18 h) 10–20 日齡東方果實蠅放入 42 cm × 35 cm × 35 cm 之壓克力箱內，30 min 後再放入陰乾之餌劑，經 4 h 後記錄死亡蟲數，以未含農藥餌劑為對照組，每處理 5 重複。(二) 乾式餌劑對東方果實蠅誘殺效果持續性試驗是將 100 隻 10–20 日齡未饑餓東方果實蠅，放入 1 m × 1 m × 1 m 之木製網籠內，經 30 min 後，分別放入暴露於戶外 1 d、1 wk、4 wk 及 8 wk 等 4 種處理的乾式餌劑 (含農藥) 及不加農藥之乾式餌劑做為對照組，於試驗進行後 4 h 記錄死亡蟲數，每處理各 5 重複。

本試驗所得各項試驗資料均以 SAS-EG (Version 4.1, 2006) 統計分析軟體進行變方分析 (analysis of variance; ANOVA) 及最小顯著差異性測驗 (least significant difference test; LSD test)。

試驗結果顯示乾式餌劑流失率隨浸水時間增加而明顯增加，但持續 24 h 浸水並未使餌劑完全溶解消失 (表 1)。經浸水 24 h 後，乾式餌劑流失率達 97.1%，但浸水過的殘餘乾式餌劑經東方果實蠅取食 4 h 後，東方果實蠅平均死亡率仍可達 99.0%。除浸水 6 h 處理組平均死亡率為 97.0% 外，其餘各處理組之東方

表 1. 餌劑經浸水處理後流失率及對東方果實蠅之毒效。

Table 1. Wash-off rates and residual toxicities of the oriental fruit fly dry-form bait after being immersed in water for various durations.

Duration of bait immersed water	% bait wash-off rate (Mean ± SE)[z]	% fruit fly mortality (Mean ± SE)[y]
1 h	4.5 ± 0.3 i[x]	99.0 ± 1.0 ab
2 h	8.7 ± 3.0 h	100.0 ± 0.0 a
3 h	12.8 ± 1.1 g	100.0 ± 0.0 a
4 h	20.7 ± 1.7 f	100.0 ± 0.0 a
5 h	27.8 ± 2.0 e	99.0 ± 1.0 ab
6 h	35.4 ± 1.4 d	97.0 ± 1.2 b
7 h	42.5 ± 2.1 d	99.0 ± 1.0 ab
8 h	52.6 ± 1.0 c	98.0 ± 2.0 ab
16 h	76.9 ± 2.0 b	98.0 ± 1.2 ab
24 h	97.1 ± 0.5 a	99.0 ± 1.0 ab
control	0.0 ± 0.0 j	100.0 ± 0.0 a

[z] Measured as (bait weight before immersing water-bait weight after immersing in water)/bait weight before immersing in water × 100.
[y] Mortality measured after the test flies feeding on the residual baits for 4 h.
[x] Means within the same column followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected LSD test; SAS-EG, Version 4.1, 2006). Percentage data were arcsine transformed prior to analysis. LSD test: least significant difference test.

果實蠅平均死亡率與未浸水乾式餌劑對照組之100.0% 死亡率間無顯著差異 (表 1)。乾式餌劑懸掛於戶外暴露 1 d、1 wk、4 wk 及 8 wk 後，對東方果實蠅平均誘殺率分別為 99.4、100.0、100.0 及 99.6%，彼此間並無顯著差異，但顯著高於未含農藥之對照組 0.0% (表 2) 。

不同的方法已用於改進東方果實蠅餌劑的耐雨及誘效持續性，如 Pinero *et al*. (2009) 使用一種模擬木瓜葉片防雨資材 [Papaya Leaf Mimic (PLM)]，將 GF-120 噴施於此資材，不僅可防雨，且 GF-120 誘效可持續至少 1 wk。Teixeira *et al*. (2009) 亦證實於 GF-120 添加石蠟可增強耐雨性，惟若要提高石蠟濃度以加強耐雨能力，則需考慮可搭配的噴施器具。不同於上述加強液體餌劑耐雨性研究，本試驗參照 Chen *et al*. (2010) 研發之乾式瓜實蠅誘殺錠，所研發之東方果實蠅乾式餌劑，本身即具耐雨特性，不似 GF-120 等液體餌劑需調製且不需搭配其它資材即可使用。本研究顯示此乾式餌劑對東方果實蠅誘殺效果可持續 8 wk；在戶外試驗 14 d 經總降雨量 110.5 mm 之沖刷，餌劑僅流失 45.4 ± 1.3%；室內毒效試驗證實此乾式餌劑經水浸泡不影響對東方果實蠅之毒效。綜合上述，東方果實蠅乾式餌劑有耐雨及誘效持久特性，具應用於田間防治東方果實蠅之潛力。

表 2. 餌劑懸掛於戶外經不同時間後對東方果實蠅之誘殺效果。

Table 2. Trapping effectiveness of bait to oriental fruit fly after exposing outdoor for different times.

Duration of bait hung outdoors	% mortality (Mean ± SE) of fruit fly attracted and killed
1 d	99.4 ± 0.4 a[z]
1 wk	100.0 ± 0.0 a
4 wk	100.0 ± 0.0 a
8 wk	99.6 ± 0.4 a
Control	0.0 ± 0.0 b

[z] Means within the same column followed by the same letter are not significantly different at $P < 0.05$ (Fisher's protected LSD test; SAS-EG, Version 4.1, 2006). Percentage data were arcsine transformed prior to analysis. LSD test: least significant difference test.

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Study on Water Resistance and Persistence of Trapping Effectiveness of an Oriental Fruit Fly Dry-Form Bait

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Abstract

Dong, Y. J. and C. C. Chen. 2014. Study on water resistance and persistence of trapping effectiveness of an oriental fruit fly dry-form bait. J. Taiwan Agric. Res. 63(3):249–252.

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a serious pest of many vegetable and fruit crops in Taiwan. This study was conducted to evaluate the water resistance and trapping effectiveness of a dry-form bait to the oriental fruit fly. Studies were conducted by immersing the baits in water or exposing the baits in the outdoors for different periods, testing the residual trapping effectiveness of the baits to the flies. The results showed that residual weights maintained about 50% after 8 h of immersion in water and its trapping effectiveness remained the same for 8 weeks. This suggests that this dry-form bait is promising for incorporation into the fruit fly integrated pest management programs.

Key words: *Bactrocera dorslais*, Bait, Water resistance, Fruit fly control, Integrated pest management.

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Phalaenopsis Sogo Yukidian ‘V3’ 蝴蝶蘭帶不同長度花梗株模擬海運日本對開花品質之影響

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摘要

黃肇家、黃慧穗、蔡金玉。2014。*Phalaenopsis* Sogo Yukidian ‘V3’ 蝴蝶蘭帶不同長度花梗株模擬海運日本對開花品質之影響。台灣農業研究 63(3):253–257。

本研究使用 *Phalaenopsis* Sogo Yukidian ‘V3’ 大白花蝴蝶蘭帶梗株為材料，植株先依梗長分為 5 組，各組梗長依次約為 1.3、2.2、3.4、4.2、5.5 cm。裝箱後，在 18℃黑暗下貯藏 11 d 以模擬海運日本。之後在一般溫室栽培，調查花梗變化與開花品質。結果發現，原始梗長較長的 (≥ 3.4 cm)，大都只有單梗，原始梗長較短的 (≤ 2.2 cm)，約 25–44% 具有雙梗，其第 2 梗大都很小，貯藏後幾乎都成為啞梗不能使用，原來的第 1 梗則都能順利正常開花。因此，在花梗數上應該可被接受。於催花時間，帶梗株貯後栽培約 60 d 即有 1 朵花開，和一般成熟株相較，可以減少催花時間約 1 mo 以上，因此可降低在日本栽培催花之成本。在開花品質上，從花梗長度 (約 50 cm)、花序長度 (約 42 cm)、花朵數 (約 12.5 朵)、花徑 (約 13 cm)、盆花壽命 (約 129 d) 來看，都是屬於極佳之品質。綜合這些結果，顯示 ‘V3’ 蝴蝶蘭帶梗株海運日本應該是可行的。

關鍵詞：蝴蝶蘭、模擬海運、花梗、盆花壽命。

前言

台灣蝴蝶蘭海運外銷已經很普遍，為促進外銷進一步發展，希望開發帶梗株之出口，出口帶梗株可以縮短在國外催花之時間 1–2 mo，對拓展外銷有很大之幫助。台灣目前在平地以冷氣催花越來越普遍，也可以在冷藏庫內以人工光照得到發梗均一之帶梗株，提高帶梗株外銷發展之潛力。

蝴蝶蘭帶梗株海運歐美需要 25–30 d 運輸時間，經模擬貯運後，部分花梗在後續栽培時不再生長成為啞梗，此問題使帶梗株海運歐美無法實用。台灣帶梗株空運出口日本已實用多年，空運成本較高，僅有少數海運，海運對帶梗株品質之影響一直未有詳細研究，本研究以模擬貯運來探討帶梗株海運日本對品質之影響及可能性。

蝴蝶蘭成熟株海運之研究甚多，包括適當之貯運溫度 (Wang 1997; Huang *et al*. 2006; Wang *et al*. 2006)，以及處理及包裝方法 (Huang *et al*. 2006)。有關蝴蝶蘭帶梗株貯運之研究很少，在國立嘉義大學之研究，顯示帶梗株比未帶梗株可以提早開花約 30–40 d，在開花品質包括花梗長度、花序長度、花朵數，帶梗株與未帶梗沒有顯著之差異 (Huang *et al*. 2010)。在行政院農業委員會農業試驗所以 10 個品種測試，發現大多數品種都有啞梗問題，啞梗率約 30–80% 依品種而異 (Huang 2011)，對開花品質影響很大，因此未能商業化使用。宜蘭大學之研究，以 LED 在貯運間照明，可以進一步縮短帶梗株貯運後之催花日數 (Chang *et al*. 2010)，也使花梗長度在貯運間大幅提高 (Chang *et al*. 2009)。LED 在貯運

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間照明也被試用於改善啞梗，大約能使啞梗發生率減少一半 (Huang 2011)，效果不夠，因此沒有實用。

材料與方法

本研究使用之植株來自台南台霖生物技術股份有限公司，為 3.5 吋大白花 (*Phal*. Sogo Yukidian 'V3')，已在該公司經過低溫催花處理，為適合外銷日本之帶梗株。

這些帶梗株送到農業試驗所之後，先置於低溫溫室 (日溫/夜溫，24–25/18–19℃) 放置 1 d。依各植株最長的花梗之梗長分組，由 0.6–1.5 cm 至 5.6–6.5 cm 等共分 5 組 (表 1)，各組梗長平均為 1.3、2.2、3.4、4.2、5.5 cm (表 2)。每組取 16 盆，分組後直立裝於未打洞之密封紙箱，紙箱長寬高各為 55 cm × 52 cm × 35 cm，每箱裝 36 株。移到 18℃，在黑暗下貯藏 11 d 以模擬海運日本。貯藏後移到一般風扇水牆溫室 (日溫/夜溫，28–30℃/23–25℃，光度 9,000–15,000 lux，約 160–270 μmol m^{-2} s^{-1}) 栽培，調查花梗之變化，到開花時，調查催花日數與開花品質。經過貯運及栽培 14 d 後 (合計 25 d)，調查梗長，若梗長沒有增加的視為啞梗，不列為梗數之內。由於在日本大白花蝴蝶蘭大都用於組盆，不需要分叉支，因此栽培過程將分叉支剪除。

催花日數為貯運後開始，到有 1 朵花開的日數。開花品質於頂端最後 1 朵花花朵盛開時調查，花梗長度為從花梗基部到最下位第 1 朵花之長度，花序長度為第 1 朵花至頂端最後 1 朵花之長度。花徑為花朵 (最下位第 2 朵) 2 個花瓣左右邊緣最大之長度。盆花壽命為植株放置在溫室，從 1 朵花開到有 1 朵花謝的日數。

表 1. *Phalaenopsis* Sogo Yukidian 'V3' 蝴蝶蘭帶梗株經裝箱後於 18℃貯藏 11 d 模擬貯運及栽培後植株梗數的變化。

Table 1. Effect of storage at 18℃ for 11 days on the spike number and abortion of the second spike of *Phalaenopsis* Sogo Yukidian 'V3' plants with spikes.

Group	Pre-storage	The existence of spike (%)					
		Pre-storage		14 d after storage		On blooming	
(Pre-storage spike length, cm)	spike number	1st spike	2nd spike	1st spike	2nd spike	1st spike	2nd spike
1 (0.6–1.5)	1.4	100.0	43.8	100.0	6.3	100.0	0.0
2 (1.6–2.5)	1.3	100.0	25.0	100.0	0.0	100.0	0.0
3 (2.6–3.5)	1.1	100.0	6.3	100.0	0.0	100.0	0.0
4 (3.6–4.5)	1.0	100.0	0.0	100.0	0.0	100.0	0.0
5 (5.6–6.5)	1.1	100.0	6.3	100.0	0.0	100.0	0.0

表 2. *Phalaenopsis* Sogo Yukidian 'V3' 蝴蝶蘭帶梗株經裝箱後於 18℃貯藏 11 d 模擬貯運及栽培後第 1 梗梗長之變化。

Table 2. Effect of storage at 18℃ for 11 days on the length of the first spike of *Phalaenopsis* Sogo Yukidian 'V3' plants with spikes.

Group	Length of the first spike (cm)			
(Pre-storage spike length, cm)	Pre-storage	After storage	14 d after storage	30 d after storage
1 (0.6–1.5)	1.3 ± 0.1[z]	2.7 ± 0.3	14.8 ± 1.1	36.3 ± 1.6
2 (1.6–2.5)	2.2 ± 0.1	4.9 ± 0.2	19.4 ± 0.5	41.2 ± 0.6
3 (2.6–3.5)	3.4 ± 0.1	6.9 ± 0.2	22.1 ± 0.5	43.3 ± 0.8
4 (3.6–4.5)	4.2 ± 0.1	7.9 ± 0.2	22.9 ± 0.6	46.1 ± 0.7
5 (5.6–6.5)	5.5 ± 0.1	9.5 ± 0.3	25.7 ± 0.8	46.6 ± 0.9

[z] Each data represents the Mean ± SE.

結果

貯運後花梗的變化

原始梗長比較短的植株，如 0.6–1.5 cm 與 1.6–2.5 cm (原始梗長 < 2.5 cm)，有 25.0–43.8% 具有雙梗 (表 1)，原始梗長較長的 (> 2.5 cm) 植株，如 2.6–3.5 cm 至 5.6–6.5 cm，幾乎都沒有雙梗，具有第 2 梗的只有 0–6.3%。

啞梗調查結果，除 0.6–1.5 cm 外，所有其他組之第 2 梗幾乎全部都沒有增長，成為啞梗 (表 3)，0.6–1.5 cm 也只有 6.3% 具有雙梗 (表 1)。後續栽培時，第 2 梗生長緩慢，和第 1 梗差距越來越大，到栽培 30 d，增長最多的為 0.6–1.5 cm，其第 2 梗也只有 5 cm (表 3)，將之去除，因此開花時，所有植株都只有單梗 (表 1)。

各植株原有之第 1 梗，在後續栽培都持續正長生長，栽培 30 d 後，長度增加約 35–40 cm (表 2)，原始梗長較長的，增加多一些。到花朵盛開時，總梗長約 90 cm (花梗長加花序長)，各組間並無顯著差異 (表 4)。

催花日數與開花品質

帶梗株經貯運後，催花日數原始花梗較長的為 57.7 d，原始花梗較短的為 63.3 d (表 4)，差距約 1 wk。整體而言，帶梗株經貯運後，催花日數大約只需 60 d。

花朵盛開時，花梗長度各組皆約 50 cm，花序長度約 42 cm，花朵數約 12.5 朵，花徑約 13 cm，各組的結果都很相近 (表 4)，盆花壽命差異也很小，原始花梗較長的為 128.5 d，

表 3. *Phalaenopsis* Sogo Yukidian ‘V3’ 蝴蝶蘭帶梗株經裝箱後於 18℃貯藏 11 d 模擬貯運及栽培後第 2 梗梗長之變化。

Table 3. Effect of storage at 18℃ for 11 days on the length of the second spike of *Phalaenopsis* Sogo Yukidian ‘V3’ plants with spikes.

Group	Length of the second spike (cm)			
(Pre-storage spike length, cm)	Pre-storage	After storage	14 d after storage	30 d after storage
1. (0.6–1.5)	0.2 ± 0.1[z]	0.4 ± 0.2	1.7 ± 1.5	4.9 ± 4.4
2. (1.6–2.5)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.7 ± 0.4
3. (2.6–3.5)	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
4. (3.6–4.5)	–[y]	–	–	–
5. (5.6–6.5)	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0

[z] Each data represents the Mean ± SE.
[y] No second spike existed.

表 4. *Phalaenopsis* Sogo Yukidian ‘V3’ 蝴蝶蘭帶梗株經裝箱後於 18℃貯藏 11 d 模擬貯運後之催花日數與開花品質。

Table 4. Effect of storage at 18℃ for 11 days on days to bloom and flower quality of *Phalaenopsis* Sogo Yukidian ‘V3’ plants with spikes.

Group (Pre-storage spike length, cm)	Days to bloom[y] (d)	Stem length[x] (cm)	Inflorescence length[w] (cm)	Number of floret	Diameter of floret (cm)	Longevity of the spike[v] (d)
1 (0.6–1.5)	63.3 ± 0.7[z]	48.7 ± 1.5	42.1 ± 1.2	12.3 ± 0.4	13.3 ± 0.1	130.1 ± 4.2
2 (1.6–2.5)	61.1 ± 0.4	49.1 ± 0.8	41.6 ± 1.0	12.4 ± 0.3	13.3 ± 0.1	132.3 ± 3.0
3 (2.6–3.5)	60.0 ± 0.5	49.9 ± 0.7	42.4 ± 0.7	12.5 ± 0.2	13.3 ± 0.0	127.8 ± 4.3
4 (3.6–4.5)	58.7 ± 0.5	48.8 ± 0.9	42.1 ± 0.7	12.6 ± 0.2	13.1 ± 0.1	128.9 ± 3.6
5 (5.6–6.5)	57.7 ± 0.3	49.9 ± 0.8	42.1 ± 1.1	12.7 ± 0.3	13.1 ± 0.1	128.5 ± 3.6

[z] Each data represents the Mean ± SE.
[y] Days to bloom represents the days after storage to the opening of the first floret.
[x] Stem length represents the length of spike from the base to the first floret at full bloom.
[w] Inflorescence length represents the length of spike from the first floret to the end of spike at full bloom.
[v] Longevity of the spike represents the days from the opening of the first floret to the senescence of any one floret. The plants were kept in greenhouse.

原始花梗較短的為 130.1–132.3 d，因此開花品質各組都很相似。

結論

‘V3’ 蝴蝶蘭帶梗株經 18℃貯藏 11 d 模擬海運日本，在花梗數上，應該是可被接受的，每棵植株最後都會有能正常開花的 1 個花梗。將一批蝴蝶蘭成熟株送進冷房催梗，於固定時間出來，必然會有梗長不同及梗數不一之帶梗株，這些帶梗株原始梗長較長的，大都只有單梗，原始梗長較短的，雙梗較高 (表 1)，這些第 2 梗大都很小。經過貯運及栽培後，具雙梗的植株，其第 2 梗幾乎全部成為啞梗，最後不能使用，但原來的第 1 梗都能順利正常開花。在日本，蝴蝶蘭單梗花，如果品質好，適合組盆，因此可被接受。

在催花時間上，帶梗株貯運後栽培約 60 d 即有 1 朵花開。通常 ‘V3’ 蝴蝶蘭成熟株，催花日數大都需要 90 d 以上，因此帶梗株海運日本可以減少在日本的催花時間約 1 mo 以上，因此可以降低在日本栽培催花之成本。

在開花品質上，本研究雖然沒有作空運或是未貯運之對照組，但是從花序長度，花朵數，花徑，盆花壽命之資料，和大部分 ‘V3’ 蝴蝶蘭未經貯運植株之開花品質相比較，此開花品質屬於極佳，因此模擬海運日本，開花品質仍是良好的。綜合這些結果，顯示 ‘V3’ 蝴蝶蘭帶梗株海運日本應該是可行的。

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Effect of Simulated Marine Shipment to Japan on the Quality of *Phalaenopsis* Sogo Yukidian 'V3' Plants with Spikes

Chao-Chia Huang[1,*], Huei-Suei Huang[2], and Chin-Yu Tsai[2]

Abstract

Huang, C. C., H. S. Huang, and C. Y. Tsai. 2014. Effect of simulated marine shipment to Japan on the quality of *Phalaenopsis* Sogo Yukidian 'V3' plants with spikes. J. Taiwan Agric. Res. 63(3):253–257.

The *Phalaenopsis* Sogo yukidian 'V3' plants with spikes were used in this study. The plants were divided into five groups according to the length of the spike. The average lengths of the spikes in each group were 1.3, 2.2, 3.4, 4.2, and 5.5 cm. All the plants were stored at 18℃ for 11 days as a simulation of marine shipment to Japan. Then they were grown in a greenhouse to observe the changes of spikes and the quality of flowers. The results indicated that the plants with longer spikes (≥ 3.4 cm) usually had only one spike in each plant. About 25% to 44% of plants with shorter spikes (≤ 2.2 cm) had two spikes. After storage all the second spike become aborted. The first spike grew normally. All the plants had one normal spike can bloom eventually. It took about 60 days for the plants with spike to have the first floret bloom after storage. It was about 30 days shorter than those plants without spike. The flower qualities of these plants were very good. At full bloom the stem length was about 50 cm, the inflorescence length was about 42 cm, the number of florets was 12.5 on each spike, and the floret diameter was about 13 cm. The longevity of the spike was about 129 days. All these data indicated that it is feasible to export *Phal*. Sogo Yukidian 'V3' plants with spike to Japan by marine shipment.

Key words: *Phalaenopsis*, Marine shipment simulation, Spike, Longevity of the spike.

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